Serious Infections in the Intensive Care Unit: diagnosis and management

Patrick Johannes van der Geest

Financial support by Astellas Pharma and Sysmex Europe for the publication of this thesis is gratefully acknowledged.

Publication of this thesis was financially supported by the department of Intensive Care Medicine of the Erasmus MC and the Erasmus University Rotterdam

ISBN: 978-94-6169-978-7

©P.J. van der Geest, The Netherlands, 2016. All rights reserved. No parts of this thesis may be reproduced or transmitted in any form or by any means, without prior permission of the author.

Layout and printed by: Optima Grafische Communicatie, Rotterdam, the Netherlands

Serious Infections in the Intensive Care Unit: diagnosis and management

Ernstige infecties in de intensive care unit: diagnose en behandeling

Thesis

to obtain the degree of Doctor from the
Erasmus University Rotterdam
by command of the rector magnificus

prof.dr. H.A.P. Pols

and in accordance with the decision of the Doctorate Board.
The public defence shall be held on

Tuesday 20 December 2016 at 9:30

Patrick Johannes van der Geest
born in Rotterdam

Erasmus University Rotterdam

DOCTORAL COMMITTEE:

Promotor:	prof.dr. A.B.J. Groeneveld
Co promotor:	dr. B.J.A. Rijnders
Other members:	prof.dr. E.C.M. van Gorp prof.dr. M.C. Vos prof.dr. D.A.M.P.J. Gommers

CONTENT OF THE THESIS

Chapter 1	General introduction and outline of the thesis	9
Section I – Prediction of infection in the critically ill:		
Chapter 2	Immature granulocytes predict microbial infection and its adverse sequelae in the intensive care unit	27
Chapter 3	The Intensive Care Infection Score – A novel marker for the prediction of infection and its severity	41
Chapter 4	The diagnostic accuracy of procalcitonin for bacteremia: a systematic review and meta-analysis	59
Chapter 5	Procalcitonin to guide taking blood cultures in the intensive care unit	89
Section II – Candida infection in the critically ill:		
Chapter 6	Safety and efficacy of amphotericin-B deoxycholate inhalation in critically ill patients with respiratory Candida spp. colonization: a retrospective analysis	111
Chapter 7	Echinocandin to fluconazole step-down therapy in critically ill patients with invasive, susceptible Candida albicans infections	127
Chapter 8	Micafungin versus anidulafungin in critically ill patients with Invasive candidiasis: a retrospective analysis	143
Section III:		
Chapter 9	General discussion	161
Chapter 10	Summary and conclusions	177
Chapter 11	Samenvatting en conclusies	185
Section IV:		
	List of Authors and Affiliations	195
	List of Publications and Presentations	199
	PhD Portfolio	201
	Curriculum Vitae	203
	Dankwoord	205

You are not defeated when you lose. You are defeated when you quit.

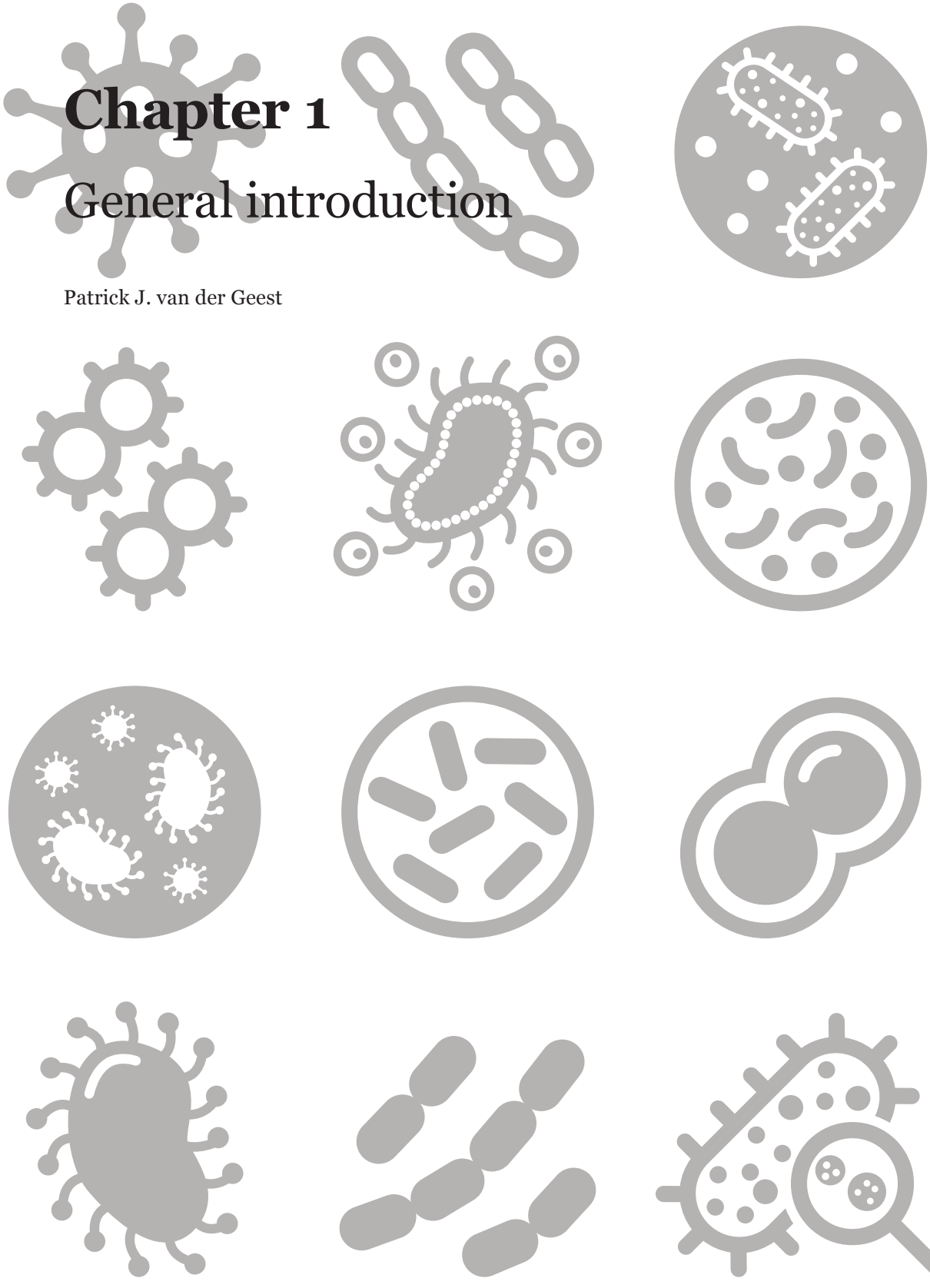
Paulo Coelho

Aan Johan†

Chapter 1

General introduction

Patrick J. van der Geest



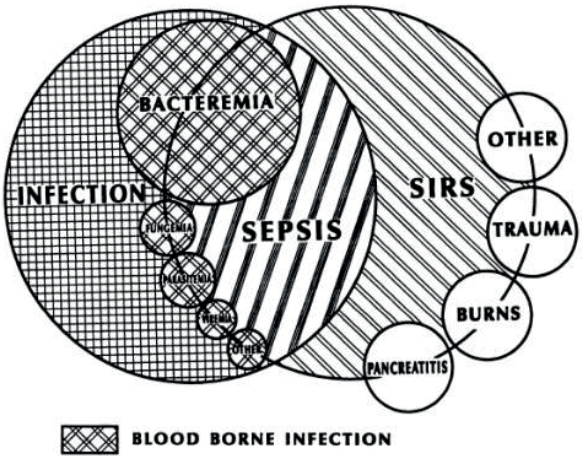
General introduction

Serious infections in the critically ill are increasing in incidence and associated with significant morbidity and mortality [1-6]. A fast and accurate diagnosis is crucial in order to reduce adverse sequelae [7]. In critically ill patients the classical signs of infection are not always present or may overlap with the symptoms of other pathological conditions (e.g. cell death after trauma or necrotizing pancreatitis). Furthermore, microbiological cultures may be difficult to obtain or can be falsely negative. All these factors make the correct diagnosis and treatment of infection in the critically ill patient challenging [7]. Therefore, there is a clear need for a fast and accurate biomarker for infection. Section I of this thesis will focus on the prediction of serious infections i.e. bloodstream infections. A substantial part of infections diagnosed in the critically ill are caused by invasive fungal infections and the incidence of invasive fungal infections is increasing [8,9]. Section II of this thesis will focus on the antifungal treatment of invasive *Candida* infections in the critically ill.

Section I– Prediction of infection in the critically ill

Because of low sensitivity and low specificity of sepsis for serious infection, the definition of sepsis has been revisited several times during the last decades to increase predictive values of infection. To assist the diagnosis of infection in clinical practice its symptoms have been grouped into the systemic inflammatory response syndrome (SIRS) [10,11]. The SIRS criteria include the following conditions: body temperature >38 °C or <36 °C; WBC (>12,000/ μL), leukopenia (<4,000/μL), or > 10% bands; heart rate >90 beats/min; and respiratory rate >20 breaths/ min or mechanical ventilation [11]. In 1992 sepsis was described as the presence of two SIRS criteria together with a probable or proven infection [11]. The SIRS criteria have prognostic value, as mortality rates increase with increasing numbers of SIRS criteria and are higher in patients with infection [4]. Of all critically ill patients with fever only around 50% will suffer from an infection [12,13]. Fever, tachycardia and tachypnea are common clinical findings in critically ill patients with non-infectious causes of SIRS [11, 14] (Figure 1). The presence of systemic inflammation indicates the possibility of an infection, but none of the SIRS criteria is specific enough to confirm the diagnosis [7]. In 2001

Figure 1. Different causes of the systemic inflammatory response syndrome.



the definition of sepsis was revisited, to optimize the prediction of infection, and included a longer list of signs of sepsis, including hemodynamic alterations, signs of organ dysfunction and the presence of inflammatory markers [15]. In 2016 these long used definitions of sepsis and septic shock were revised again, because the SIRS- based definition was too non-specific, and considerable advances have been made into the understanding of the pathophysiology, the management, and the epidemiology of sepsis [16] (Table 1). The definition of sepsis has been revised several times, and the use of the sepsis syndrome as a surrogate for proven infection as an outcome parameter may be too sensitive and nonspecific. Therefore, we have chosen to study the prediction of serious infections (i.e. bacteraemia) in this thesis, which is a more robustly defined culture proven infection.

Diagnosing serious infections in critically ill patients is not always straightforward [7]. Critically ill patients often have ongoing disease problems, which can induce signs of inflammation without the presence of infection [7]. Furthermore, most critically ill patients are on antibiotics, which can result in false negative microbial cultures. Therefore, the need for more specific and accurate biomarkers is necessary to discriminate infection from non-infectious causes of SIRS in the critically ill patient.

Table 1.

- Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.
- Organ dysfunction can be identified as an acute change in total sequential organ failure assessment (SOFA) score ≥2 points consequent to the infection.
- Patients with suspected infection who are likely to have a prolonged ICU stay or to die in the hospital can be promptly identified at the bedside with qSOFA (quick SOFA), i.e., alteration in mental status, systolic blood pressure ≤100 mm Hg, or respiratory rate ≥22/min.
- Patients with septic shock can be identified with a clinical construct of sepsis with persisting hypotension requiring vasopressors to maintain MAP ≥65 mm Hg and having a serum lactate level >2 mmol/L (18 mg/dL) despite adequate volume resuscitation.

SOFA score

	Score				
System	0	1	2	3	4
Respiration					
Pao_2/Fio_2, mm Hg (kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7) with respiratory support	<100 (13.3) with respiratory support
Coagulation					
Platelets, $\times 10^3/\mu L$	≥150	<150	<100	<50	<20
Liver					
Bilirubin, mg/dL (μmol/L)	<1.2 (20)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (204)
Cardiovascular	MAP ≥70 mm Hg	MAP <70 mm Hg	Dopamine <5 or dobutamine (any dose)[b]	Dopamine 5.1-15 or epinephrine ≤0.1 or norepinephrine ≤0.1[b]	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1[b]
Central nervous system					
Glasgow Coma Scale score[c]	15	13-14	10-12	6-9	<6
Renal					
Creatinine, mg/dL (μmol/L)	<1.2 (110)	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440)	>5.0 (440)
Urine output, mL/d				<500	<200

Abbreviations: Fio_2, fraction of inspired oxygen; MAP, mean arterial pressure; Pao_2, partial pressure of oxygen.

[a] Adapted from Vincent et al.[27]

[b] Catecholamine doses are given as μg/kg/min for at least 1 hour.

[c] Glasgow Coma Scale scores range from 3-15; higher score indicates better neurological function.

During the last years many biomarkers have been investigated as potential predictors of infection. To discuss all investigated biomarkers in this introduction would be impossible and therefore we will focus on the most promising and most frequently used biomarkers nowadays. C-reactive protein (CRP) is an acute phase protein produced by the liver, which will be released just a few hours after inflammation is caused by variable conditions [17]. Another potential biomarker, which has been suggested to be more reliable than CRP, is procalcitonin (PCT) [18]. Procalcitonin is a peptide precursor of the hormone calcitonin, which rises in a response to inflammation especially caused by infection [19]. Both markers are frequently determined and widely available, but the predictive values vary and both markers are also elevated in non-infectious conditions of

inflammation [18, 20-25]. Like all other biomarkers they have in common that the predictive values are only moderate and they are more useful to rule out disease than to pinpoint infection [7]. This latter property of PCT could be useful to exclude the need for culturing blood in critically ill patients with fever. PCT has a high negative predictive value for the presence of microbiologically proven infection, so that a normal PCT level (PCT <0.25 ug/L) could be used to rule out the presence of bacteraemia in different clinical settings [26].

Sepsis is the result of a very complex host immune response to infection and therefore it will be unlikely that a single maker will ever be useful to detect infection [7, 15]. An option would be to combine different biomarkers, whether or not with clinical data, into a scoring system [27-29]. Another option would be the use of proteomics or genomics for the prediction of infection [30]. At last, but behind the scope of this thesis, new techniques that allow for the simultaneous detection of e.g. DNA, RNA and cell wall components in body fluids (e.g. blood, sputum, broncho-alveolar lavage) are looking promising, but to what extend they will be able to improve patient outcome remains to be seen [31]. Classical blood cultures need at least 12 hours to become positive and only after another day or more full identification with an antibiogram will be available. Furthermore, in many critically ill patients with sepsis blood cultures remain negative [32]. PCR technology is able to detect DNA traces of common bacterial and fungal infections in just a few hours [31]. Giving the complexity of proteomics, genomics and PCR techniques and the costs involved in this new area of research, it will be more likely that a new and more reliable scoring system for the prediction of infection will be developed in the next years. Important considerations for the development of such a scoring system are to determine which clinical parameters and which biomarkers should be included, using standardized assays, clear identification of cut-offs, and more homogeneous groups, in order to make the comparison of different scoring systems easier.

Questions to be answered in this thesis:

1. Are immature granulocytes useful for the prediction of infection and it's sequelae in critically ill patients? Should this marker replace other commonly used markers or should it be used together with the other markers? To answer this question, **Chapter 2** of this thesis will present the results of a retrospective study analysing the predictive value of Immature Granulocytes compared with the white blood cell count and C-reactive protein for microbial infection in critically ill patients. The study was performed in 46 patients critically ill patients.

2. Is the Intensive Care Infection Score an useful score for the prediction of infection in critically ill patients? In **Chapter 3** of this thesis we tried to answer this question by presenting the results of a prospective multi-center study on the predictive value of a new scoring system, the Intensive Care Infection Score (ICIS), for microbial infection in critically ill patients. The ICIS score was compared with the most frequently used biomarkers nowadays. The study included 301 patients.

3. Should we use procalcitonin as a marker for the prediction of bacteraemia or should we use it as a marker to exclude bacteraemia? In **Chapter 4** of this thesis we tried to answer this question by presenting the results of a systemic review and meta-analysis on the diagnostic accuracy of procalcitonin for bacteraemia. The study included 58 articles, representing 16,514 patients.

4. Can procalcitonin be used to guide decision making on blood sampling for cultures in critically ill patients? In **Chapter 5** of this thesis we tried to answer this question by evaluating the usefulness of procalcitonin as a decision tool to sample (or not) blood for culture in critically ill patients. The ProBIC study is a prospective, multicentre, cluster-randomized, cross-over trial and included 564 patients.

Section II – Candida infection in the critically ill

The incidence of serious infections in the critically ill continuous to increase [1-6]. Over the last decades the share of invasive fungal infections and in particular *Candida* spp. among the ICU patient with infection increased as well [8,9]. Invasive fungal infections are associated with prolonged hospital stay and increased mortality [8,9]. Early treatment with an appropriate antifungal drug is mandatory since mortality of candidemia is directly correlated with delayed antifungal therapy [33.34].

In the Netherlands critically ill patients receive selective decontamination of the digestive tract (SDD) when the expected duration of mechanical ventilation is more than 48 hours. SDD involves the administration of the non-absorbable antimicrobial agents tobramycin, amphotericin-B and colistin. Patients also receive cefotaxim 1 gram intravenously four times a day for a three-day period. As part of this protocol, inventory cultures are taken of the throat, tracheal aspirates, and rectum on admission. To monitor the effect of SDD treatment, surveillance cultures (from throat, tracheal aspirates, and rectum) are performed routinely done thrice weekly as well. When the SDD cultures from the tracheal aspirate are repetitively positive for fungal growth (i.e. colonization), the SDD protocol recommends to start amphotericin-B deoxycholate (ABDC) inhalation therapy via a nebulizer (Table 2). Treatment with ABDC can then be stopped after two consecutive cultures of the respiratory tract are negative or after detubation of the patient [35]. The use of ABDC to treat respiratory colonization with *Candida* spp. is still a subject of debate. First, *Candida* spp. are frequently cultured from non-sterile body sites in critically ill patients, but the clinical significance hereof is not easily defined [36-40]. When *Candida* spp. are repetitively cultured from the respiratory tract it mostly reflects harmless colonization, for which the start of antifungal treatment is a subject of debate, but in some rare cases it could also reflect an invasive pulmonary fungal infection or may be a source of a subsequent candidemia [36-40]. Second, the safety and efficacy of ABDC treatment is not well established, and its use as an aerosol can interfere with pulmonary surfactant, a molecule produced by type II alveolar cells and essential for efficient exchange of gases and for maintaining the structural integrity of alveoli [41-44].

Table 2. Description of the different types of antifungal agents.

Polyene antimycotics Polyene antimycotics are obtained from some species of *Streptomyces* bacteria. The polyenes bind to ergosterol in the fungal cell membrane and cause leakage of K+ and Na+ ions, which contributes to fungal cell death. Examples of polyene antimycotics are: amphotericin-B deoxycholate and nystatin. They can be administered intravenously or via inhalation.
Azoles Azoles are antifungal drugs that inhibit the fungal enzyme 14α-demethylase which produces ergosterol (an important component of the plasma fungal membrane). The azoles in clinical use for systemic therapy are: fluconazole, voriconazole, isavuconazole, posaconazole and itraconazole. They can be given intravenously or via the oral route.
Echinocandins Echinocandins are a relatively novel class of antifungal agents with activity against a broad spectrum of *Candida* spp., including *C. Glabrata* and *C. Krusei*, against which fluconazole has less or no activity. They inhibit the synthesis of glucan in the cell wall, via non-competitive inhibition of the enzyme 1,3-β glucan synthase. At this moment there are three echinocandins in clinical use: caspofungin, anidulafungin and micafungin. They can be administered intravenously only.

Table 3. Definitions of invasive fungal infections.

Candidemia Defined as at least one positive blood culture for *Candida spp*. drawn from a peripheral vein or a central venous catheter.
Intra-abdominal candidiasis Intra-abdominal specimens (obtained surgically, through a sterile puncture or a drain that is in place for <24 h) positive for *Candida* spp., irrespective of the fungal concentration and associated bacterial growth. Or a positive culture with *Candida* spp. obtained from a normally sterile site, such as peritoneal fluid.
Invasive pulmonary candidiasis Positive cultures with *Candida* spp. obtained from a normally sterile site, such as pleural fluid, in the context of pleural exsudate/empyema. Or the presence of yeast cells in a lung biopsy.

In case a patient is suspected of having an infection, additional cultures (besides the routinely performed SDD cultures) can be taken from the possible source of infection, which includes the use of bloodcultures. A suspicion of infection can be raised by the presence of one or more of the following signs and symptoms of infection: fever or hypothermia; hypotension; localized signs and symptoms of inflammation; or radiological signs of an infection. When the cultures from sterile body sites become positive for fungal growth, treatment with an appropriate antifungal agent is necessary. In Table 3 the definitions of invasive fungal infections are given. Guidelines recommend to start with an echinocandin for the treatment of proven invasive fungal infections caused by *Candida* spp. in critically ill patients [45]. There are three echinocandins in clinical use which can be chosen for therapy (Table 2). All three echinocandins are

considered equally effective but studies that compared these different echinocandins directly in critically ill patients with a proven invasive candida infection are scarce [46]. However, differences between the in vitro antifungal activity, the pharmacokinetic and toxicity profiles exist, but the clinical relevance is probably small[47]. The treatment duration is based on recommendation made by guidelines, which take several factors in account, such as the duration of positive cultures, the certainty of good drainage and clinical improvement, including absence of fever for >24 hours and haemodynamic stability, and recovery from neutropenia [45,48,49]. The recommended minimum length of treatment for a bloodstream infection caused by candida (candidemia) is 14 days after the first negative culture [45,48]. Removal of intravenous catheters which are a possible or likely source of the candidemia as well as drainage of suspected pus collections are important therapeutic interventions in the treatment of invasive candida infection as well.

During antifungal treatment it is possible to step-down to an azole in patients who have improved clinically after initial treatment with an echinocandin and in whom azole susceptible *Candida* spp. have been documented [45,48]. The timing of the step-down is subject of debate, guidelines suggest to step-down after at least 10 days of initial treatment with echinocandin, while others suggest that this could be done earlier [45,48,50].

Questions to be answered in this thesis:

1. Is amphotericin-B inhalation therapy a safe and effective treatment for Candida spp. colonization of the respiratory tract? In **Chapter 6** of this thesis we tried to answer this question by evaluating the safety and efficacy of amphotericin-B deoxycholate inhalation as a treatment for respiratory colonisation with *Candida* spp. in critically ill patient. This retrospective study was performed in 113 patients.

2. Can we step-down to fluconazole safely after initial treatment with an echinocandin for invasive and susceptible *C. albicans* in the critically ill? To answer this question we studied the safety and efficacy of step-down therapy from an echinocandin to an azole in critically ill patients with invasive *Candida*

albicans infections in **Chapter 7** of this thesis. This retrospective study was performed in 56 patients.

3. Is micafungin safe and effective for the treatment of invasive candidiasis in critically ill patients? In **Chapter 8** of this thesis we tried to answer this question by comparing the safety and efficacy of micafungin with anidulafungin for the treatment of invasive *Candida* spp. infections in critically ill patients. This retrospective study was performed in 63 patients.

Aim and outline of the thesis

The main content of this thesis is divided into two sections. In section I we studied the predictive values of different biomarkers for serious infections i.e. bloodstream infections. The first goal was to find a biomarker that could discriminate between non-infectious causes of inflammation and microbial infection. The second goal was to determine if one of those biomarkers could predict the invasiveness (bacteraemia) and severity (septic shock, mortality) of microbial infection. The third goal was to determine optimal cut-off values for the prediction of microbial infection.

In the section II we describe the safety and efficacy of different antifungal treatment strategies in critically ill patients. The goal was to evaluate the safety and efficacy of currently used antifungal agents and strategies. The second goal was, when possible, to give recommendations for possible changes in the current antifungal treatment strategies. These two sections are followed by a commentary that summarizes and discusses the main findings.

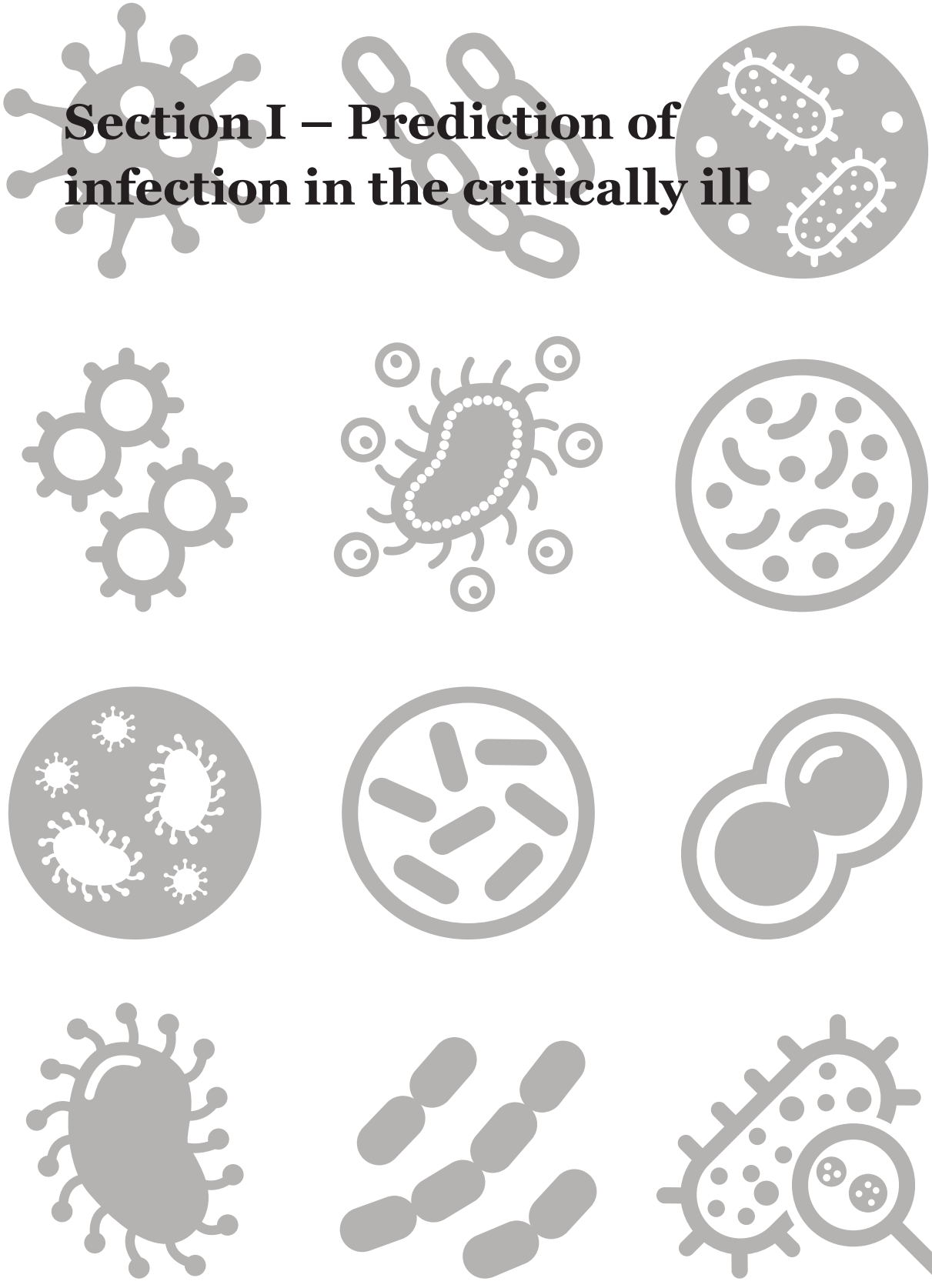
References

1. Angus DC, Linde-Zwirble WT, Lidicker J, et al. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001; 29: 1303-10.
2. Van Gestel A, Bakker J, Vervaart CP, et al. Prevalence and incidence of severe sepsis in Dutch intensive care units.
3. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006;34:1589-96.
4. Vincent JL, Sakr Y, Sprung CL, et al. Sepsis in European intensive care units: results of the SOAP study.. Crit Care Med 2006; 34: 344-53.
5. Wang HE, Szychowski JM, Griffin R, et al. Long-term mortality after community-acquired sepsis:a longitudinal population-based cohort study. BMJ Open 2014;4:e004283.
6. Goncalves-Pereira J, Pereira JM, Ribeiro O, et al. Impact of infection on admission and of the process of care on mortality of patients admitted to the Intensive Care Unit – The INFAUCI study. Clin Microbiol Infect. 2014; 20: 1308-153.
7. Vincent JL, Beumier M. Diagnostic and prognostic markers in sepsis. Expert Rev Anti Infect Ther 2013; 11: 265-275
8. Kett DH, Azoulay E, Echeverria PM, Vincent JL. Candida bloodstream infections in intensive care units: analysis of the extended prevalence of infection in intensive care unit study. Crit Care Med 2001; 39: 665-670.
9. Glockner A. Treatment and prophylaxis of invasive candidiasis with anidulafungin, caspofungin and micafungin – review of the literature. Eur J Med Res 2011; 16: 167-179.
10. Bone RC, Fisher CJ jr, Clemmer TP, et al. Sepsis syndrome: a valid clinical entity. Methylprednisolone severe sepsis study group. Crit Care Med 1989; 17: 389-393.
11. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992;101:1644-55.
12. Circiumaru B, Baldock G, Cohen J. A prospective study of fever in the intensive care unit. Intensive Care Med 1999; 25: 668-673.
13. Peres Bota D, Lopes Ferreira F, Mélot C, et al. Body temperature alterations in the critically ill. Intensive Care Med 2004; 30: 811-816.
14. Dulhunty JM, Lipman J and Finfer S. Does severe non-infectious SIRS differ from severe sepsis? Intensive Care Med 2006; 34: 1654-1661.
15. Levy MM, Fink MP, Marshall JC, et al. SCCM/ ESICM/ ACCP/ ATS/ SIS International sepsis definitions conference. Crit Care Med 2011; 31: 1250-1256.
16. Mervyn Singer, Clifford S Deutschman, Crostopher Warren Seymour. The third international consensus definitions for sepsis and septic shock (sepsis -3). JAMA 2016; 315: 801-810.
17. Vincent JL, Donadello K and Schmitt X. Biomarkers in the critically ill patient: C-reactive protein. Critical Care Clinics 2011; 27: 241-251.
18. Luzzani A, Polati E, Dorizzi R, et al. Comparison of procalcitonin and C-reactive protein as markers of sepsis. Crit Care Med 2003; 31: 1737-1741.
19. Riedel S. Procalcitonin and the role of biomarkers in the diagnosis and management of sepsis. Diagnostic Microbiol and Inf Dis 2012;73:221-227.
20. Charles PE, Kus E, AHO S, et al. Serum procalcitonin for the early recognition of nosocomial infection in the critically patients: a preliminary report. BMC Infect Dis 2009; 9: 49.

21. Kibe S, Adams K, Borlow G. Diagnostic and prognostic biomarkers of sepsis in critical care. J Antimicrob Chemother 2011; 66: i33-ii40.
22. Vincent JL, Donadello K, Schmit X. Biomarkers in the critically ill patient: C-reactive protein. Crit Care Clin 2011; 27: 241-251.
23. Wacker C, Prkno A, Brunkhorst FM, et al. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect Dis 2013; 13: 426-435.
24. Bloos F, Reinhart K. Rapid diagnosis of sepsis. Virulence 2014; 5: 154-160.
25. Garnacho-Montero J, Huici-Moreno MJ, Gutiérrer-Pizarraya A, et al. Prognostic and diagnostic value of eosinopenia, C-reactive protein, procalcitonin and circulating cell-free DNA in critically ill patients admitted with suspicion of sepsis. Crit Care 2014; 18: R116
26. Sandra Hoeboer, Patrick J van der Geest, Daan Nieboer, et al. The diagnostic accuracy of procalcitonin for bacteraemia: a systematic review and meta-analysis. Clin Microbiol Infect 2015; 21: 474-481.
27. Peres Bota D, Melot C, Lopes Ferreira F, et al. Infection probability score (IPS): A method to help assess the probability of infection in critically ill patients. Crit Care Med 2003; 31: 2579-2584.
28. Kofoed K, Andersen O, Kronborg G, et al. Use of plasma C-reactive protein, procalcitonin, neutrophils, macrophage migration inhibtory factor, soluble urokinase-type plasminogen activator receptor, and soluble triggering receptor expressed on myeloid cells-1 in combination to diagnose infection: a prospective study. Crit Care 2007; 11: R38.
29. Andaluz-Ojeda D, Bobillo F, Iglesias V, et al. A combined score of pro- and anti-inflammatory interleukins improves mortality prediction in severe sepsis. Cytokine 2012; 57: 332-336.
30. Feezor RJ, Cheng A, Paddock HN, et al. Functional genomics and gene expression profiling sepsis: beyond class prediction. Clin Infect Dis 2005; 7: S427-S435.
31. Dark PM, Dean P, Warhurst G. Bench-to-bedside review: the promise of rapid infection diagnosis during sepsis using polymerase chain reaction-based pathogen detection. Crit Care 2009; 13: 217.
32. Ratzinger F, Schuardt M, Eichbichler K, et al. Utility of sepsis biomarkers and the infection probability score to discriminate sepsis and systemic inflammatory response syndrome in standard care patients. Plose One 2013; 8: e82946.
33. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of candida bloodstream infection until positive bloodculture results are obtained: a potential risk factor for hospital mortality. Antimicrob Agents Chemother 2005; 49: 3640-3645.
34. Garey KW, Rege M, Pai MP, et al. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi institutional study. Clin Infect Dis 2006; 45: 25-31.
35. CP Stoutenbeek, HK van Saene, DR Miranda, et al. The effect of selective decontamination of the digestive tract on colonisation and infection rate in multiple trauma patients. Intensive Care Med 1984; 10: 185–192.
36. El-Ebiary M, Torres A, Fabregas N, et al. Significance of the isolation of candida species from the respiratory samples in critically ill, non-neutropenic patients: an immediate post- mortem histologic study. Am J Respir Crit Care Med 1997; 156: 583-590.
37. Rello J, Esandi ME, Diaz E, et al. The role of Candida sp isolated from bronchoscopic samples in non-neutropenic patients. Chest 1998; 14:146-149.
38. Charles PE, Dalle F, Doise JM, et al. Candida spp. colonization significance in critically ill medical patients; a prospective study. Intensive Care Med 2005; 31: 393-400.
39. Eggimann P, Calandra T, Fluckiger U, et al. Invasive candidiasis: comparison of management choices by infectious disease and critical care specialists. Intensive Care Med 2005; 31: 1514-1521.
40. Delisle M-S, Williamson DR, Perreault MM, et al. The clinical significance of Candida colonization of respiratory tract secretions in critically ill patients. J Crit Care 2008; 23: 11-17.

41. Ong DS, Klein Klouwenberg P, Spitoni C, et al. Nebulised amphotericin B to eradicate Candida colonisation from the respiratory tract in critically ill patients receiving selective digestive decontamination: a cohort study. Crit Care 2013; 17: R233.
42. Van 't Veen A, Gommers D, Mouton JW, et al. Exogenous pulmonary surfactant as a drug delivering agent: influence of antibiotics on surfactant activity. Br J Pharmacol 1996; 118: 593-598.
43. Ruijgrok EJ, Vulto AG, Van Etten WM. Efficacy of aerosolized amphotericin B desoxycholate and liposomal amphotericin B in the treatment of invasive pulmonary aspergillosis in severely immunocompromized rats. J Antimicrob Chemother 2001; 48: 89-95.
44. Knechtel SA, Klepser ME. Safety of aerolized amphotericin B. Expert Opin Drug Saf 2007; 6: 523-532.
45. Pappas PG, Kauffman CA, Andes D, et al. Clinical guidelines for the management of candidiasis: 2009 update by the infectious diseases society of America. Clin Infect Dis 2009; 48: 505-535.
46. Kullberg BJ, Verweij PE, Akova M, et al. European expert opinion on the management of invasive candidiasis in adults. Clin Microbial Inf 2011; 5: 1-12.
47. Pappas PG, Coleman M, Rotstein F, et al. Micafungin versus caspofungin for treatment of candidemia and other forms of invasive candidiasis. Clin Infect Dis 2007; 45: 883-893.
48. Cornely OA, Bassetti M, Calandra T, et al. ESCMID guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. Clin Microbiol Inf 2012; 18: 19-37.
49. Reboli AC, Shorr AF, Rotstein C, et al. Anidulafungin compared with fluconazole for treatment of candidemia and other forms of invasive candidiasis caused by Candida albicans: a multivariate analysis of factors associated with improved outcome. BMC Inf Dis 2011; 11: 261.
50. Vazquez J, Reboli AC, Pappas PG, et al. Evaluation of an early step-down strategy from intravenous anidulafungin to oral azole therapy for the treatment of candidemia and other forms of invasive candidiasis: results from an open-label trial. BMC Inf Dis 2014; 14: 97.

Section I – Prediction of infection in the critically ill



Chapter 2

Immature granulocytes predict microbial infection and its adverse sequelae in the intensive care unit

Patrick J. van der Geest,[1] Mostafa Mohseni,[1] Rob Brouwer,[2] Ben van der Hoven,[1] Ewout W. Steyerberg[3] and A.B. Johan Groeneveld.[1]

[1]Department of Intensive Care medicine, Erasmus MC, Rotterdam, the Netherlands.
[2]Department of Clinical Chemistry, Erasmus MC, Rotterdam, the Netherlands.
[3]Department of Public Health, Erasmus MC, Rotterdam, the Netherlands.

Journal of Critical Care 2014; 29(4): 523-7.

Abstract

Background

We evaluated the predictive value of immature granulocyte (IG) percentage in comparison with white blood cell counts (WBC) and C-reactive protein (CRP), for infection, its invasiveness and severity in critically ill patients.

Methods

In 46 consecutive patients, blood samples were collected at the day (0) of a clinical suspicion of microbial infection and at day 1 and 3 thereafter. We defined infections, bloodstream infection (BSI) and septic shock within 7 days after enrolment.

Results

Of the 46 patients, 31 had infection, of whom 15 developed BSI and 13 septic shock. CRP and IG percentage increased with increasing invasiveness and severity of infection, from day 0 onwards. Receiver operating characteristic analysis (ROC) to predict infection showed an area under the curve (AUC) of 0.66 (P=0.10) for WBC versus 0.74 (P=0.01) for CRP and 0.73 (P=0.02) for IG percentage on day 0. Comparing WBC and CRP to WBC and IG percentage results in comparable prediction of microbial infection. Comparing WBC and CRP with WBC, CRP and IG percentage suggests an additional early value of IG percentage, when not elevated, in ruling out infection. No marker carried any prognostic significance.

Conclusion

IG percentage is a useful marker, as CRP, to predict infection, its invasiveness and severity, in critically ill patients. However, the IG percentage adds to WBC and CRP in the early exclusion of infection, and can be obtained routinely without extra blood sampling or costs.

Introduction

Only about 50% of clinically suspected infections in the intensive care unit (ICU) can be confirmed by imaging techniques and microbiological cultures[1,2]. Leucocytosis with neutrophilia and increasing bands in the peripheral blood (indicating a left shift in the differentiation) are classical hallmarks of infection but their diagnostic accuracy vary, particularly in the critically ill, so that the search for better markers, including C-reactive protein (CRP), is ongoing[2-4]. Notwithstanding, international guidelines recommend to use a band count greater than 10% as a criterion for systemic inflammatory response syndrome and sepsis[3].

The quantification of immature granulocytes (IG) in the white blood cell count (WBC) differential count is routinely done by visual microscopy, which is labour intensive and time consuming but still considered helpful, particularly in leukopenic patients[5]. Nowadays, the differential count of WBC can be automated in modern cell counting machines. This allows simple, fast and inexpensive quantification of the IG promyeloytes, myelocytes and metamyelocytes, as a marker of bone marrow activity, without the need for taking extra blood samples[1,6-9]. The total number of IG is expressed both as absolute cell count and as a percentage of WBC, i.e. the IG percentage, as supplied by the Sysmex XE-2100[4,10,11]. The latter has been studied as a predictor of neonatal sepsis and bloodstream infection[12,13]. The IG percentage may also be elevated in adult (non-critically ill) patients with (bloodstream) infection[8,11,14] or, less well defined, sepsis, particularly when severe[6,9]. There is one study in 70 adult critically ill surgical patients, who may have multiple reasons for developing a left shift in WBC, showing that an increased IG percentage helped to predict infection, superior to CRP[4]. Another study on the critically ill suggested the IG percentage to better help predict sepsis with a suspected or proven infection than CRP[1], which is less helpful in predicting proven infection. The value of IG percentage in addition to conventional infection markers (such as CRP) remained unclear in many of the foregoing studies, as well as its relation to invasiveness (bloodstream versus local infection) and severity of infection, e.g. development of hemodynamic and coagulation derangements and attributable mortality[2,4,6,9].

We therefore studied the predictive value of the IG percentage, in comparison to WBC count and CRP, in patients with a clinically suspected infection in the ICU. The hypothesis was that the IG percentage is of additive value in predicting microbial infection, its invasiveness and severity.

Patients and methods

Patients

Informed consent was waived of for this study because the study parameters were collected during routine assessment, without extra blood sampling, of critically ill patients with a suspicion of infection. Data were treated anonymously, so that according to Dutch law no consent is required. The study was performed in a consecutive 5 months period. We included all consecutive patients admitted into the ICU with a clinical suspicion of infection on the day of admission on the basis of body temperature >38 °C or <36 °C measured rectally while in the ICU, a WBC count >10,000/μL or <4,000/μL or CRP >9 mg/L. Patients with neutropenia (WBC count <0.5/μL) or immunodeficiency (HIV, solid cancer, hematologic malignancy, solid- organ or bone marrow transplantation, or long term or high dose steroid treatment) were excluded.

Clinical protocol

Patients were taken care of by intensivists unaware of the study results. The clinical suspicion of infection led to appropriate imaging and culturing at the discretion of attending intensivists, starting at day 0 of the study. A routine chest radiograph was obtained at day 0 and other imaging examinations were ordered when considered appropriate. Cultures were taken from different sources (blood, tracheal aspirate, urine, draining fluids), in order to confirm infection and were reported by the medical microbiological laboratory. Infection was defined and managed by the attending intensivist on the basis of clinical, imaging and microbiological data in close collaboration with infectious disease specialists and according to published criteria[15], within 7 days after enrolment. We routinely use selective decontamination of the digestive tract (SDD) for patients longer than 48 h on mechanical ventilation and 72 h in the ICU. This involves administration of an oral paste and of a suspension via the nasogastric tube, containing the non-absorbable antibiotics tobramycin, amphotericin-B and colistin.

Study protocol and data collection

At day 0 of study enrolment when the patient was suspected for having an infection, baseline demographic data and clinical variables, including age, sex, pre- morbidity, prior use of antibiotics, including SDD, steroids, immune status

(active malignancy or other causes of an immunocompromised state), reasons of admission, use of mechanical ventilation, use of renal replacement therapy were recorded. The acute physiology and chronic health evaluation II (APACHE II) was calculated on admission. On day 0, 1 and 3 clinical data were recorded, such as temperature, heart rate and respiratory parameters taken from the ventilator. The primary site of infection, blood culture results, presence of sepsis or septic shock and use of norepinephrine during a 7 day observational period after enrolment were recorded. Blood samples for the analysis of WBC, CRP and IG percentage were routinely obtained at days 0, 1 and 3. Blood samples were collected from an arterial catheter, routinely in place in our ICU patients. The WBC was measured using a Sysmex XE-5000 analyzer (Toa Medical Instruments, Kobe, Japan), CRP (using an Immunoturbidimetric assay, Modular Analytics <P> Roche diagnostics, Mannheim, Germany).The IG percentage was calculated with a specific type of automatic cell analyzer the Sysmex XE-2100 Hematology analyzer (Toa Medical Instruments, Kobe, Japan). The method is described in detail elsewhere[16]. In brief, the IG measurement, which includes promyelocytes, myelocytes, and metamyelocytes but not bands or blasts, is performed in the differential channel. A lysing reagent causes disruption of mature WBC membranes, leaving bare nuclei, while immature myeloid cells with low cell membrane lipid content remain intact. The increased permeability of leukocytes allows a polymethine dye to enter the cells with high affinity for nucleic acid. Subsequently, the cells are analyzed by nucleic acid fluorescence and side scatter. The IG percentage is the percentage of IG of total WBC count. Detection of IG by the Sysmex XE-2100 as compared to flow cytometry and previously automated and manual counting showed high correlation and predictive values, suggesting excellent performance[10,12,17-20]. The upper limit of normal in blood samples from healthy volunteers is 0.4% (mean of 0.22%)[19]. Patients were followed up until day 28 after enrolment and length of ICU stay was recorded.

Definitions

The systemic inflammatory response syndrome (SIRS) was defined as two or more of the following conditions: (a) body temperature >38 °C or <36 °C; (b) WBC (>10,000/ μL), leukopenia (<4,000/μL), or >10% bands; (c) heart rate >90 beats/min; and (d) respiratory rate >20 breaths/ min or mechanical ventilation. Sepsis was defined when meeting two or more of the SIRS criteria, together with a

confirmed microbial infection within 7 days after suspicion of infection was raised. Bloodstream infection (BSI) was defined as having a positive blood culture with a recognized pathogen except skin contaminants[15]. Shock was defined as an acute circulatory failure characterized by persistent systolic arterial pressure <90 mm Hg or mean arterial pressure (MAP) <70 mm Hg for at least one hour despite adequate fluid resuscitation or requirement of vasopressor support to maintain MAP. In the presence of sepsis, shock was considered septic shock.

Statistical analysis

Continuous variables were expressed as the mean with standard deviation (SD) or, when the assumption of normality was violated (Kolmogorov- Smirnov test $P<0.05$), as median values and interquartile ranges. More than 2 groups were compared with help of the Kruskal-Wallis test for non-normally distributed data; otherwise the Student's t test was used. For categorical data, the Fisher exact test was used. Areas under the receiver operating characteristics curves (AUCROC) curves were calculated to evaluate predictive values. The additive value of IG percentage above WBC and CRP for the prediction of infection was assessed with Harrell's c- statistics (c- index which is identical to ROC) and the net reclassification improvement (NRI) by using R v 2.14 with the package for multivariate imputation by chained equations (MICE, R Foundation for Statistical Computing, Vienna, Austria) for SPSS version 20.0.0[21]. The NRI attempts to quantify how many individuals are correctly reclassified when adding a new marker, the IG percentage, to the old markers WBC and CRP. Since clearly defined classification thresholds were not available, the continuous variant of NRI was used. All tests were two-sided and a $P<0.05$ was considered statistically significant. Exact P values >0.001 are given.

Results

Patients and infections

Table 1 describes the characteristics of the 46 consecutive patients enrolled in the study of whom 31 had a clinical suspicion of infection confirmed. Most patients were admitted to the ICU after elective surgery; only 2 patients were admitted because of suspected infection. Patients were enrolled at a median of 4

(5) days after ICU admission. Patients with an infection more often had a history of diabetes mellitus than patients without infection. The APACHE II score was higher and the use of renal replacement therapy was more frequent in patients with infection. Since all patients met SIRS criteria, the patients with infection had sepsis and 13 developed septic shock, 2 (2.5) days after enrolment. Table 2 shows that BSI was diagnosed at median day 4 after enrolment in 15 patients. A total of 10 species of microorganism were detected from microbial cultures. Mortality rates did not differ between infection groups (Table 1).

Table 1. Patient characteristics.

	Infection	No infection	P
	n=31	n=15	
Age (years)	18 (58)	9 (60)	0.54
Gender male)	59 (32)	53 (21)	0.90
APACHE II (day1)	16 (6)	11 (8)	0.002
Premorbidity			
Cardiac	6 (19)	3 (20)	0.96
Pulmonary	7 (23)	1 (7)	0.12
Renal	4 (13)	2 (13)	0.97
Neurological	5 (16)	2 (13)	0.81
DM	4 (13)	0 (0)	0.04
Intestinal	20 (65)	10 (67)	0.89
Cancer	12 (39)	8 (53)	0.36
Immune	1 (3)	1 (7)	0.60
Other	4 (13)	2 (13)	0.97
Reasons of ICU admission			0.74
Postoperative	23 (77)	10 (66)	
Trauma	4 (13)	3 (20)	
Hepatic dysfunction	1 (3)	1 (8)	
Neurological	1 (3)	1 (7)	
Suspected infection	2 (7)	0	
Septic shock	13 (42)	0	0.002
CVVH	6 (19)	0	0.01
Mechanical ventilation	23 (74)	10 (67)	0.61
Norepinephrine	24 (77)	2 (13)	0.001
Antibiotics	31 (100)	8 (53)	0.001
Length of stay ICU (days)	18 (51)	6 (3)	0.001
28-day mortality	15 (48)	4 (27)	0.16

Numbers (percentage) or median (interquartile range), where appropriate. Abbreviations: APACHE = Acute Physiology and Chronic Health Evaluation II; DM = diabetes mellitus; CVVH = continuous venovenous haemofiltration; ICU = intensive care unit.

Markers

Table 3 shows that CRP and IG percentage increased with increasing invasiveness of microbial infection on day 0, 1 and 3. In patients with infection who developed septic shock the IG percentage at day 3 was higher than in patients with infection only (Table 4). No marker carried any prognostic significance.

Predictive values

The AUROC for the prediction of infection on day 0 was 0.66 (P=0.10) for WBC versus 0.74 (P=0.01) for CRP and 0.73 (P=0.02) for IG percentage. The AUROC to predict BSI on day 0 was 0.53 (P=0.74) for WBC versus 0.76 (P=0.005) for CRP and 0.79 (P=0.002) for IG percentage. Only day 3 AUROC's for WBC (0.74, P=0.014) and IG percentage (0.82, P=0.001) helped to predict septic shock (ROC curves can be found in the online supplement). Table 5 summarizes predictive values.

We combined variables and judged their additive values. Comparing model 1 (WBC and CRP) to model 2 (WBC and IG percentage) results in comparable AUROC for the prediction of microbial infection (Table 6). The predictive

Table 2. Infection characteristics.

	n	Days between enrolment and diagnosis
Source (may be more than one)		
Bacteremia	15	4 (2)
Pneumonia	15	2.5 (2.5)
Peritonitis	3	2 (2.5)
Soft tissue	3	2 (2.5)
Miscellaneous	5	1.5 (2)
Associated microorganism		
Staphylococcus aureus	15 (19)	
Pseudomonas spp.	14 (18)	
Klebsiella pneumoniae	11 (14)	
Enterococcus spp.	11 (14)	
Candida spp.	10 (13)	
Escherichia coli	10 (13)	
Proteus mirabilis	3 (4)	
Streptococcus pneumoniae	2 (3)	
Stenotrophomonas spp.	1 (2)	
Morganella morganii	1 (2)	

Numbers (percentage) or median (interquartile range), where appropriate.

value for absence of infection improves for each day when IG percentage is added (model 3) to WBC and CRP (model 1) (Figure 1). For day 3, the IG percentage improved the NRI for both having an infection and not having an infection. When combining IG percentage with the WBC and CRP, the sensitivity of the model 3 was 80% at 90% specificity and the specificity was 75% at 90% sensitivity.

Table 3. Infection markers in patients with infection and bloodstream infection (BSI), patients with infection but no BSI or patients without infection**.**

	Day	Infection and BSI n=15	Infection, no BSI n=16	No infection n=15	P
WBC, 10^9/L	0	9.6 (11.0)	12.6 (7.1)	8.9 (6.1)	0.19
	1	11.8 (4.8)	13.4 (7.9)	9.2 (5.5)	0.02
	3	11.3 (9.2)	11.9 (7.7)	7.1 (2.9)	0.02
CRP, mg/L	0	239 (247)	111 (67)	80 (65)	0.008
	1	306 (152)	170 (168)	96 (114)	< 0.001
	3	245 (140)	106 (176)	89 (84)	0.006
IG percentage	0	1.3 (1.7)	0.4 (0.7)	0.2 (0.2)	0.003
	1	1.1 (2.4)	0.6 (0.8)	0.4 (0.6)	0.006
	3	1.8 (2.7)	1.1 (1.5)	0.3 (1.1)	0.009

Data are given as median (interquartile range). WBC = white blood cell count, CRP = C-reactive protein, IG = immature granulocytes.

Table 4. Infection markers in patients with or without septic shock.

	Day	Shock n=13	No shock n=18	P
WBC, 10^9/ L	0	11.3 (4.8)	10.6 (6.4)	0.96
	1	12.5 (5.1)	12.9 (5.4)	0.71
	3	14.0 (6.1)	10.4 (5.2)	0.07
CRP, mg/ L	0	192 (120)	159 (119)	0.56
	1	243 (118)	211 (114)	0.45
	3	212 (111)	160 (95)	0.35
IG percentage	0	2.7 (5.0)	1.0 (1.4)	0.27
	1	3.3 (5.9)	1.5 (2.9)	0.24
	3	3.5 (2.7)	1.3 (1.1)	0.01

Mean (standard deviation). WBC = white blood cell count, CRP = C-reactive protein, IG = immature granulocytes.

Table 5. Individual predictive indices for infection for day 0 markers.

Cut-off value	WBC	CRP	IG percentage
	>12.6 10^9/L	>99 mg/L	>0.4 %
Sensitivity	45	77	58
Specificity	93	71	80
PPV	93	85	86
NPV	45	59	48

WBC = white blood cell count, CRP = C-reactive protein, IG = immature granulocytes, PPV = positive predictive value, NPV = negative predictive value.

Table 6. Net reclassification improvement (NRI).

	Day 0	Day 1	Day 3
c- index (model 1)	0.75	0.82	0.81
c- index (model 2)	0.75	0.80	0.85
P	0.79	0.49	0.25
NRI infection, P	- 0.03, P = 0.59	- 0.22, P = 0.20	0.10, P = 0.59
NRI no infection, P	- 0.09, P = 0.80	0.06, P = 0.80	0.20, P = 0.44
NRI overall, P	0.07, P = 0.92	- 0.16, P = 0.60	0.30, P = 0.35
c- index (model 1)	0.75	0.82	0.81
c- index (model 3)	0.80	0.87	0.88
P	0.01	0.01	0.001
NRI infection, P	- 0.03, P = 0.86	- 0.03, P = 0.86	0.23, P = 0.21
NRI no infection, P	0.60, P = 0.02	0.60, P = 0.02	0.47, P = 0.07
NRI overall, P	0.57, P = 0.07	0.57, P = 0.07	0.69, P = 0.03

The concordance (c) statistic (identical to ROC and p for their comparison is given for model 1 (WBC and CRP), model 2 (WBC and IG percentage) and model 3 (WBC, CRP and IG percentage) at day 0, 1 and 3. The NRI and associated P are calculated for infection, no infection and overall reclassification.

Discussion

This study suggests that the IG percentage is an easily obtainable and useful adjunctive marker to predict microbial infection, its invasiveness and severity in the critically ill patient.

The elevation of IG percentage in patients with infection is confirmed in earlier studies, in (non-)critically ill adults and neonates[1,7,11,12,14]. One of those studies concluded that the IG percentage better predicted infection than the total WBC count, in agreement with our study, but similarly as the absolute neutrophil count[11]. ROCs performed in our study showed AUROCs between 0.66-0.73 for the WBC and 0.73-0.78 for IG percentage, indicating that the

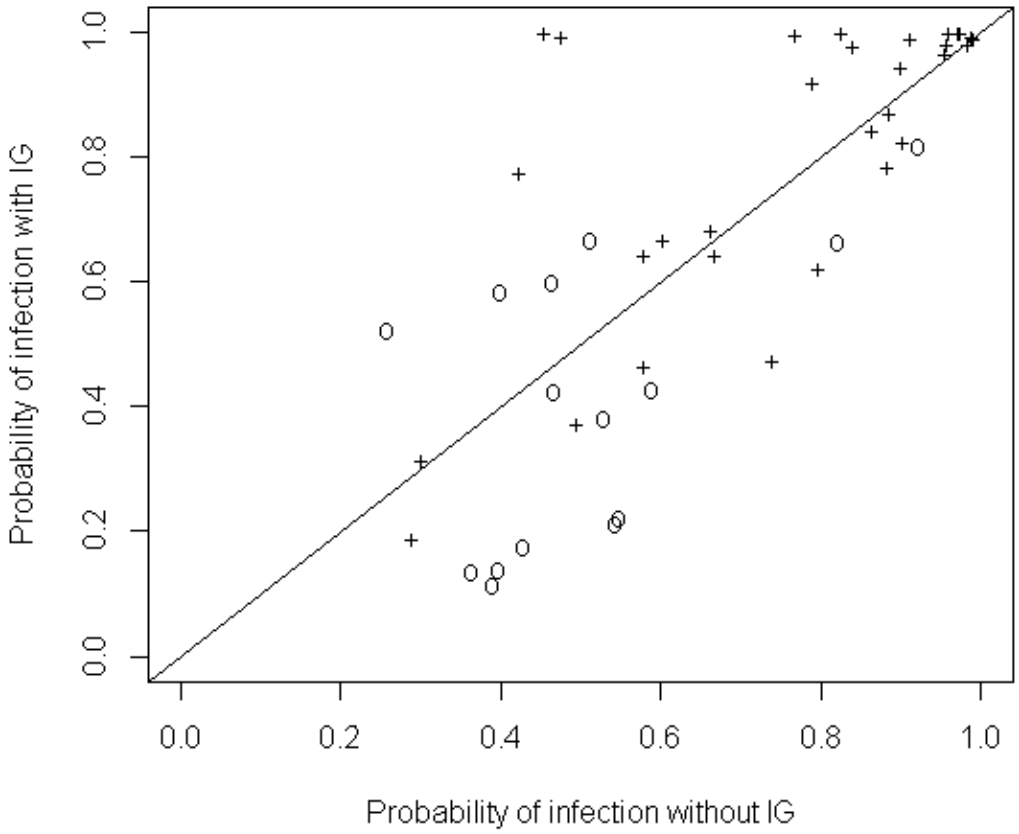


Figure 1. Reclassification plot of infection (+) and no infection (o) when IG percentage is added to WBC and CRP at day 0. It is shown that the addition particularly lowered the probability of no infection.

IG percentage is indeed a better predictor of infection. Other studies suggest that IG percentage can be used as indicator for the invasiveness and severity of microbial infection[1,7,8,11,14], also in line with our study. The AUROC for IG percentage to predict infection, BSI and septic shock, and thus the invasiveness and severity of infection is similar or higher than reported in other studies in non-critically ill patients[11,12,14]. Of the two studies performed in the critically ill, the IG percentage was used to predict otherwise loosely defined sepsis, rather than proven infection[1]. Our results largely agree with those obtained in critically ill surgical patients including the absent relation with mortality[4], which may not agree with other studies showing IG to predict mortality[7].

Studies tried to combine IG percentage with WBC, absolute neutrophil count or other markers to increase the accurate prediction of microbial infec-

tion[1,4,5,7,8,11,14]. The IG percentage combined with the WBC was as predictive as WBC combined with CRP, so that the IG percentage can replace CRP, even in the critically ill, as suggested by our study. In fact, the predictive values of IG percentage and CRP as single markers were similar. Adding IG percentage to WBC and CRP gave a better prediction of microbial infection (AUC 0.80-0.88) than using the IG percentage as a single marker, and its addition was helpful at day 0 to exclude infection and at day 3 to predict or exclude infection. Hence, the IG percentage can still be useful when CRP measurements are available.

Furthermore, the IG percentage can be rapidly and accurately generated with each full blood count at no extra cost, as opposed to the use of other available markers for infection including CRP. The automatic measurement of IG is more precise and less labor intensive than the manual differential count, resulting in improved quality and even cost reduction in the clinical chemistry laboratory[9,12,17,20]. Because the measurement can be performed on the same machines incorporated for the daily routine measurements of the WBC count, no additional blood sampling is needed either.

A limitation of this study is the relatively small population enrolled in this study, so that results should be regarded as preliminary. There is a significant difference in disease severity between infected and non-infected patients, so that we cannot rule out whether the same alterations in IG percentage could be seen when both groups were comparably sick. The use of SDD may not interference with the predictive value of infection markers as concluded by Hoeboer et al[2]. The current study did not compare IG percentage with other novel biomarkers such as procalcitonin (PCT). However a PCT measurements requires extra blood sampling and additional costs in contrast to the IG percentage which can be obtained routinely without extra blood sampling nor costs. An advantage is the use of the relatively novel NRI indicating the additive value of IG percentage in addition to WBC and CRP for the prediction of infection. This was not performed in previous studies.

In conclusion, the IG percentage is a useful marker, as CRP, to predict infection, its invasiveness and severity, in the critically ill patient. However, the IG percentage adds to WBC and CRP in early exclusion of infection, and can be collected routinely without extra blood sampling or costs. The value of IG percentage in guiding infection management and improving patient outcomes deserves further study.

References

1. Park BH, Kang YA, Park MS et al. Delta neutrophil index as an early marker of disease severity in critically ill patients with sepsis. BMC Infect Dis 2001; 11:29.
2. Hoeboer SH, Alberts E, Van den Hul I et al. Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: a case for procalcitonin. J Infect 2012; 64:484-493.
3. Cavallazzi R, Bennin C-L, Hirani A, et al. Is the band count useful in the diagnosis of infection? An accuracy study in critically ill patients. J Intensive Care Med 2010; 25:353-357.
4. Nierhaus A, Klatte S, Linssen J et al.Revisiting the white blood cell count: immature granulocytes count as a diagnostic marker to discriminate between SIRS and sepsis – a prospective, observational study. BMC Immunology 2013; 14:8.
5. Wile MJ, Homer LD, Gaehler S et al. Manual differential counts help predict bacterial infection. A multivariate analysis. Am J Clin Pathol 2001; 115:644-649.
6. Nahm CH, Choi JW, Lee J. Delta neutrophil index in automated immature granulocyte counts for assessing disease severity of patients with sepsis. Ann Clin Lab Sci 2008; 38:241-246.
7. Simson E, Groner W. The state of the art for the automated WBC differentials. Lab Hematol 1995; 1:13-22.
8. Park D-H, Park K, Park J et al. Screening of sepsis using leukocyte cell population data from the Coulter automatic blood cell analyzer DxH800. Internat J Lab Hematol 2011; 33:391-399.
9. Seok Y, Choi JR, Kim J et al. Delta neutrophil index: a promising diagnostic and prognostic marker for sepsis. Shock 2012; 37:242-246.
10. Briggs C, Harrison P, Grant, et al. New quantitative parameters on a recently introduced automated blood cell counter: the XE-2100. Clin Lab Haematol 2000; 22:345-350.
11. Briggs C, Kunka S, Fujimoto H et al. Evaluation of immature granulocyte counts by the XE-IG master: upgraded software for the XE-2100 automated hematology analyzer. Lab Hematol 2009; 9:117- 124.
12. Nigro KG, O'Riordan M, Molloy EJ et al. Performance of an automated immature granulocyte count as a predictor of neonatal sepsis. Am J Clin Pathol 2005; 123:618–624.
13. Bender L, Thaarup J, Varming K et al. Early and late markers for the detection of early-onset neonatal sepsis. Dan Med Bull 2008; 55:219–223.
14. Senthilnayagam B, Kumar T, Sukumaran J, et al. Automated measurement of immature granulocytes: performance characteristics and utility in routine clinical practise. Pathol Res Internat 2012; 2:483-489.
15. Calandra T, Cohen J. International sepsis forum definition of infection in the ICU consensus conference. Crit Care Med 2005; 33:1538-48.
16. Ali Ansari-Lari M, Kickler TS, Borowitz MJ. Immature granulocyte measurement using the Sysmex XE-2100. Am J Clin Pathol 2003; 120:795-799.
17. Ruzicka K, Veitl M, Thalhammer- Scherrer R, et al. The new hematology analyzer Sysmex XE-2100: performance evaluation of a novel white blood cell differential technology. Arch Pathol Lab Med 2001; 125:391-396.
18. Weiland T, Kalkman H, Heihn H. Evaluation of the automated immature granulocyte count (IG) on Sysmex XE-2100 automated haematology analyser vs. visual microscopy (NCCLS H20-A). Sysmex J Int 2002; 12:63–70.
19. Bruegel M, Fiedler GM, Matthes G et al. Reference values for immature granulocytes in healthy blood donors generated on the Sysmex XE-2100 automated hematology analyser. Sysmex J Int 2004; 14: 5-7.
20. Fernandes B and Hamaguchi Y. Automated enumeration of immature granulocytes. Am J Clin Pathol 2007; 128:454- 463
21. Van Buuren S, Groothuis-Oudshoorn K. Mice: multivariate imputation by chained equations in R. J Stat Software 2011; 45:1-67.

Chapter 3

The Intensive Care Infection Score – A novel marker for the prediction of infection and its severity

Patrick J. van der Geest,[1] Mostafa Mohseni,[1] Jo Linssen,[2] Servet Duran,[3] Robert de Jonge[4] and A.B. Johan Groeneveld.[1]

[1] Department of Intensive Care Medicine and Department of Clinical Chemistry[4] of the Erasmus Medical Center, Rotterdam, The Netherlands.

[2] Faculty of Health Science, University of Medicine, Institute of Immunology, University Witten/ Herdecke, Witten, Germany.

[3] Department of Intensive Care Medicine of the Maasstad Hospital, Rotterdam, The Netherlands.

Critical Care2016;20:180 .

Abstract

Introduction

The prediction of infection and its severity remains difficult in the critically ill. A novel, simple biomarker derived from five blood-cell derived parameters that characterize the innate immune response in routine blood samples, the intensive care infection score (ICIS), could be helpful in this respect. We therefore compared the predictive value of the ICIS with that of the white blood cell count (WBC), C-reactive protein (CRP) and procalcitonin (PCT) for infection and its severity in critically ill patients.

Methods

We performed a multi-center, cluster randomized, crossover study in critically ill patients between January 2013 and September 2014. Patients with a suspected infection for which blood cultures were taken by the attending intensivist were included. Blood was taken at the same time for WBC, ICIS, CRP and PCT measurements in the control study periods. Results of imaging and cultures were collected. Patients were divided into groups of increasing likelihood of infection and invasiveness: Group 1 without infection or with possible infection irrespective of cultures, Group 2 with probable or microbiologically proven local infection without blood stream infection (BSI) and Group 3 with BSI irrespective of local infection. Septic shock was assessed.

Results

In total, 301 patients were enrolled. CRP, PCT and ICIS were higher in Group 2 and 3 than 1. The AUROC for the prediction of infection was 0.70 for CRP, 0.71 for PCT and 0.73 for ICIS ($P<0.001$). For the prediction of septic shock the AUROC was 0.73 for CRP, 0.85 for PCT and 0.76 for ICIS. These AUROC's did not differ from each other.

Conclusion

The data suggest that the ICIS is potentially useful for the prediction of infection and its severity in critically ill patients, non-inferiorly to CRP and PCT. In contrast to CRP and PCT, the ICIS can be determined routinely without extra blood sampling and lower costs, yielding results within 15 minutes.

Introduction

In approximately 50% of critically ill patients with suspected infection an infection is probable or can be proven [1,2]. This could lead potentially to overtreatment with empiric antibiotics [1,2]. Moreover, infection diagnostics are often delayed since it takes 48 h at minimum for cultures to become positive and thereby to prove a clinically suspected infection. Hence, there is a continuing need for fast and accurate biomarkers of infection, that may help to predict infection, its invasiveness and severity and may guide empiric antibiotic treatment in the future [3]. We belief that prediction of infection is more helpful in patient management than prediction of sepsis, since the majority of critically ill patients have two or more criteria of the systemic inflammatory response syndrome (SIRS) as criteria for sepsis, irrespective of infection.

Commonly applied biomarkers include C-reactive protein (CRP) and procalcitonin (PCT), but predictive values vary among studies [3-11]. A new biomarker is the Intensive Care Infection Score (ICIS) which is composed of five blood cell-derived parameters characterizing the early innate immune response and routinely obtainable in blood samples sent to the laboratory for cell counts. The ICIS score has been retrospectively evaluated in two pilot studies on 70 and 172 patients, respectively, suggesting potential predictive value for infection [12,13].

We therefore performed a prospective study on the predictive value of ICIS for probable or proven infection in critically ill patients with suspected infection, and compared its performance with that of the white blood cell count (WBC), CRP and PCT levels. We hypothesized that in the critically ill patient with suspected infection the diagnostic accuracy of the simply obtainable ICIS is at least equivalent in this respect to WBC, CRP and PCT, without requiring extra blood sampling.

Methods

Study design and patients

The ICIS study is an add-on non-interventional study on patients which had been enrolled into a prospective, cluster-randomized, cross-over trial, involving both intensive care units (ICU) of the Erasmus Medical Center Rotterdam and

both ICU's of the Maasstad hospital Rotterdam. The ICU's were stratified and randomized by treatment regimen into a control group (standard of care) and an intervention group. In the intervention arm, blood culturing for a suspected infection was guided by PCT measurements. The acronym PCT-guided blood culturing in the intensive care, ProBIC, was used for this study and results will be reported later. The trial was conducted between January 2013 and September 2014. The ICU of the Erasmus Medical Center is a tertiary care mixed medical-surgical ICU with approximately 2000 admissions per year. The ICU of the Maasstad hospital is a secondary care mixed medical-surgical ICU with 1200 admissions per year. The trial was conducted in accordance with the ethical principles decreed by the Declaration of Helsinki and in compliance with International Conference on Harmonization Good Clinical Practice Guidelines. The institutional review board (IRB) or the independent medical ethical committee at each of the investigational centers (Medisch Ethische commissie Maasstad ziekenhuis, Rotterdam, Nederland and Medisch Ethische commissie Erasmus Medisch Centrum, Rotterdam, Nederland) reviewed and approved the protocol, amendments and informed consent document. The medical ethical committee of the Erasmus Medical Center finally approved the study (MEC 2011-505). The trial is registered at ClinicalTrial.gov (protocol ID NCT01847079) on 24 April 2013. All patients or their proxy provided written informed consent prior to study inclusion, at ICU admission. Inclusion criteria were age above 18 and below 80 years and the clinical suspicion of infection for which the attending intensivist established a medical need for a blood culturing. Suspicion of infection included but was not limited to increased body temperature above 38.3 °C (tympanic temperature), chills, progressive leukocytosis, increased CRP, increasing consolidations on chest radiography or other imaging of potential infection sources. For each patient it was possible to be included more than once, but we analyzed for the current study only the first time that blood for culture was taken. Patients were excluded if they were pregnant, had neutropenia (defined as leukocyte count less than $0.5x10^9$/L), used immunosuppressive or immunostimulatory therapy, or had a predetermined illness with an expected death within 24 hours. Patients were not included if blood cultures were performed as part of a standard protocol (such as patients with veno-venous or veno-arterial extra corporeal membrane oxygenation) or were performed to check the effectiveness of treatment (such as in endocarditis), unless the blood culture was done because of suspicion of

infection. The ICU's switched the allocated regimen every three months, so that there were six three-months episodes of standard care in which 774 patients were eligible for inclusion and 473 patient were excluded (≤ 18 years 5; neutropenia ($<0.5x10^{4/}$ L) 63; uncontrolled malignancy 35; immunosuppressive medication 256; suspect dead < 24 hours 22; no informed consent 92). Data for the ICIS study were thus collected in 301 patients in the control arm (six, three months episodes) of the ProBIC study.

Study protocol, data collection and assays

Baseline demographic data and clinical variables were recorded at the day of inclusion, and included age, sex, comorbidity, reasons of admission, use of antibiotics including selective decontamination of the digestive tract (SDD), antifungal treatment, steroids, immunosuppressive medication, immune status and recent surgery. The treatment received during ICU stay was also recorded and included mechanical ventilation, renal replacement therapy, total parenteral nutrition, arterial and central venous catheters and the use of vasopressor or inotropic medication. The acute physiology and chronic health evaluation II (APACHE II) and the sequential organ failure assessment (SOFA) score were recorded at admission. The length of ICU and hospital stays and vital outcomes were recorded, up to 90 days after inclusion. At the same time that blood for culture was taken for each, blood samples for determinations of WBC, CRP, PCT and ICIS (Day 0) were taken. Blood for similar measurements (except for PCT) was taken in the morning of the two following days (Day 1 and 2). Treating physicians and investigators were blinded for the PCT and ICIS measurement results. Also the outcome adjudicators that decided presence/ absence of infection were blinded to biomarker results. Two sets of blood cultures were taken and directly send to the department of medical microbiology. The set taken for blood culture consists of one aerobic and one anaerobic bottle (BD Bactec™, New Yersey, USA) that contain resin to enhance recovery of organisms. The samples were incubated for a seven-day period in an automatic analyzer (BD BACTEC™, New Jersey, USA) that automatically demonstrates the time to positive blood culture in case of positive bacterial or fungal growth. Gram strains were performed, and the organisms were cultured on agar plates after growth identification was performed, using the VITEK® 2 (Biomerieux, Marcy l'Etoile, France). Blood for the WBC and ICIS measurement was obtained in a K3EDTA tube. Both the

WBC and ICIS parameters were measured on a modified fluorescence flow hematology analyzer with fully automated gating (Sysmex, Kobe, Japan) [14]. The ICIS was measured promptly after collection but within a maximum of 24 hours. The ICIS score is composed of five blood-cell derived parameters that characterize the innate immune response [15-19]. The five parameters include the mean fluorescence intensity of mature (segmented) neutrophils, the difference in hemoglobin concentration between newly formed and mature red blood cells, the total segmented neutrophil count, the antibody secreting lymphocytes and the accurate immature granulocytes count, as previously described [12]. Each parameter is available from a standard routine method and can be measured within 1 minute without sample preparation on a modified fluorescence flow hematology analyzer with fully automated gating (Symex, Kobe, Japan) [12]. The methodology is based on routine hematology fluorescence flow cytometry using different fluorescence reagents for mainly nucleic acids, as well as specifically designed blood cell membrane surfactant reagents generating information about cell shape and the formation of bioactive lipids from cell membranes [12]. Side and forward scatter light are used to determine intracellular structure and size of blood cells [12]. By adding all weighting values for all five parameter components the maximum possible ICIS score is 20. Serum CRP (turbidimetric assay) and PCT (electrochemiluminescence BRAHMS immunoassay) measurements were routinely performed using an Cobas 8000 platform (Roche, Almere, Netherlands). Blood for PCT measurement was sampled in a z serum clot activator tube.

Definitions

After completion of the study, the investigators decided whether an infection was present from Day 0-2, on the basis of the available imaging and culture results. The outcome adjudicators were blinded for all biomarkers. Source and likelihood of infection were based on criteria defined at the International Sepsis Forum Consensus Conference [20]. For judging results of cultures, a 48 h window prior and after taking blood cultures was taken. The causative microorganisms were recorded. Blood stream infection (BSI) was defined as a positive blood culture with a recognized pathogen except skin contaminants [20,21]. In case of skin contaminants, BSI was considered if at least 2 blood cultures drawn on separate locations were positive [20,21]. Patients were divided into groups

of increasing likelihood of infection and invasiveness of associated micro-organisms, suggestive of increasing severity: Group 1 without infection or with possible infection irrespective of cultures, Group 2 with probable (irrespective of cultures) or proven local infection (with positive cultures of a causative microorganism) without BSI and Group 3 with BSI irrespective of local infection. SIRS was defined as two or more of the following criteria: (I) body temperature >38 °C or <36 °C; (II) WBC (>10,000/μL), leukopenia (<4,000/μL), or >10% bands; (III) heart rate >90 beats/min; and (IV) respiratory rate >20 breaths/min or mechanical ventilation, for values at Day 0. When SIRS and a probable/proven infection (Group 2 or 3) were present, patients were classified as having sepsis. Shock was defined as an acute circulatory failure characterized by persistent systolic arterial pressure <90 mm Hg or mean arterial pressure (MAP) <70 mm Hg for at least one hour despite adequate fluid resuscitation or requirement of vasopressor support to maintain MAP, at Day 0. In the presence of sepsis, shock was considered septic shock.

Statistical analysis

This was performed using SPSS version 23 (SPSS inc., Chicago Ill., USA) and using R package. Data are expressed are expressed as median (interquartile range) or as number of patients (percentage) where appropriate. Most data were distributed non-normally (Kolmogorov-Smirnov test $P<0.05$). Group (>2) differences were evaluated using the Kruskal-Wallis test or X^2 test, for continuous and categorical data, respectively. The Mann-Whitney U test and Fisher exact test were used to compare two groups. To evaluate predictive values we calculated the areas under the receiver operating characteristic curves (AUROC) for Day 0 values. For the predictive values of sepsis and septic shock we used the values of Day 0. We consider an AUROC >0.70 as clinically relevant [22]. The optimum cut-off value was calculated on the basis of the highest sensitivity and specificity combined (Youden index). Positive and negative predictive values were calculated. To correct for multiple testing we set the level of statistical evidence at $P\leq0.01$. Exact P values >0.001 are given.

Results

Patient characteristics

Table 1 describes the baseline characteristics of the 301 patients enrolled: 149 (Group 1) patients had no infection and 152 (Group 2+3) patients had a probable or proven infection. Patients with a probable or proven infection were older and more often had a history of cancer, cardiac disease or gastro-intestinal problems. Mechanical ventilation or renal replacement therapy was more often used in patients with a probable or proven infection. All patients with a probable or proven infection were on antibiotics. No difference was seen in 28- or 90-day mortality and in the length of ICU or hospital stay .

Source of infection and microbial species

The abdomen and lungs were the most frequent source of infection (Table 2). Gram-positive pathogens were mostly cultured, followed by Gram-negatives, fungi and viruses (Table 2).

Biomarkers

Table 2 shows the infection markers according to invasiveness of infection. Most patients had SIRS on Day 0, so that the patients with infection in Group 2 and 3 had mostly sepsis. CRP, PCT and ICIS were increased at Day 0-2 in patients with infection as compared to those without infection. In contrast to PCT, there was no difference in CRP and ICIS between Groups 3 and 2. The CRP, PCT and ICIS where increased in patients with septic shock (Table 3).

Predictive values

The AUROC for the prediction of infection (Group 2+3 vs Group 1) on Day 0 was similar for CRP, PCT and ICIS (Table 4, Fig. 1). At a cut-off ≥7, the positive predictive value of ICIS was >80% and at a cut-off ≤1 the negative predictive value of ICIS was >80%. Otherwise, the AUC for ICIS did not differ from that of any other biomarkers, including PCT, expect for that of WBC ($P<0.001$). PCT had the highest AUROC for the prediction of septic shock (AUROC 0.85, $P<0.001$), but not different from that of ICIS (AUROC 0.76, $P<0.001$), whereas CRP had an AUROC of 0.73 ($P<0.001$) and WBC of 0.53 ($P=0.68$) (Fig. 2).

Table 1. Baseline demographic and clinical characteristics.

	Group 1 (n=149)	Group 2+3 (n=152)	P
Age (years)	57 (24)	62 (19)	0.01
Gender (male)	100 (67)	105 (69)	0.72
APACHE II score	22 (10)	22 (8)	0.92
APACHE IV score	63 (38)	60 (34)	0.49
SOFA score	7 (7)	8 (6)	0.06
Comorbidity			
Neurologic	39 (26)	41 (27)	0.88
Cardiac	40 (27)	58 (38)	0.04
Pulmonary	28 (19)	38 (25)	0.25
Gastrointestinal	39 (26)	55 (36)	0.05
Renal	15 (10)	24 (16)	0.14
DM II	22 (15)	33 (22)	0.13
Cancer	22 (15)	46 (30)	0.002
Autoimmune	7 (5)	8 (5)	0.97
Reasons of ICU admission			<0.001
Suspected infection	23 (15)	69 (45)	
Respiratory failure	24 (16)	33 (22)	
Renal failure	0 (0)	1 (1)	
Liver failure	5 (3)	3 (2)	
Neurology	31 (21)	8 (5)	
CPR	9 (6)	5 (3)	
Shock	10 (7)	3 (2)	
Trauma	13 (9)	5 (3)	
Postoperative	34 (23)	25 (17)	
Treatment on ICU			
Antibiotics	140 (94)	152 (100)	0.02
Norepinephrine	109 (73)	128 (84)	0.03
Dobutamine	18 (12)	17 (11)	0.80
TPN	39 (26)	50 (33)	0.20
Mechanical ventilation	133 (89)	125 (82)	0.06
Renal replacement therapy	15 (10)	52 (34)	<0.001
Length of ICU stay (days)	9 (17)	11 (17)	0.49
Length of hospital stay (days)	22 (34)	26 (36)	0.13
Mortality day 28	42 (28)	58 (38)	0.09
Mortality day 90	98 (36)	68 (45)	0.13

Numbers (percentage) or median (interquartile range), where appropriate. Abbreviations: APACHE II = Acute Physiology and Chronic Health Evaluation II; CPR = cardiopulmonary resuscitation; DM II = diabetes mellitus type II; ECMO= extra corporeal membrame oxygenation; SOFA = sequential organ failure assessment score; TPN = total parenteral nutrition.

Table 2. Infection characteristics.

	Group 1	Group 2	Group 3	**P**
	(n=149)	(n=91)	(n=61)	
Source of infection				0.01
Pulmonary	-	44 (48)	15 (25)	
Abdominal	-	30 (33)	29 (47)	
Urogenital	-	8 (9)	2 (3)	
Neurologic	-	2 (2)	2 (3)	
Soft tissue/ bones	-	7 (8)	10 (17)	
Blood and catheter	-	0	3 (5)	
Gram strain				0.01
Gram negative	-	30 (33)	16 (26)	
Gram positive	-	27 (30)	36 (59)	
Type of microorganism				0.15
Staphylococci	-	15 (16)	17 (28)	
Streptococci	-	12 (13)	19 (31)	
Enterobacteriaceae	-	26 (29)	15 (25)	
Pseudomonas	-	4 (4)	1 (2)	
Fungi	-	12 (13)	6 (10)	
Viral	-	5 (5)	3 (5)	
Biomarkers				
SIRS	146 (98)	90 (99)	58 (95)	0.94
Septic shock	-	12 (13)	9 (15)	0.78
Temperature (°C)	38.1 (1.5)	38.3 (1.7)	38.0 (1.5)	0.94
Heart rate (beats/ min)	105 (29)	109 (37)	112 (39)	0.15
Respiratory rate (breaths/ min)	28 (13)	29 (17)	29 (17)	0.61
WBC day 0 (10^9/L)	12.4 (7.8)	14.4 (12.9)	13.9 (12.2)	0.63
WBC day 1 (10^9/L)	11.7 (8.1)	13.9 (13.1)	13.4 (11.3)	0.47
WBC day 2 (10^9/L)	12.1 (7.2)	14.4 (9.8)	14.8 (15.0)	0.19
CRP day 0 (mg/L)	84 (109)	163 (156)	167 (161)	<0.001
CRP day 1 (mg/L)	88 (131)	156 (156)	197 (154)	<0.001
CRP day 2 (mg/L)	82 (141)	131 (136)	180 (172)	<0.001
PCT day 0 (µg/L)	0.65 (2.30)	2.71 (9.88)	4.13 (38.0)	<0.001
ICIS day 0	3 (3)	6 (5)	6 (5)	<0.001
ICIS day 1	3 (3)	6 (4)	6 (4)	<0.001
ICIS day 2	4 (4)	6 (4)	6 (4)	<0.001

Numbers (percentage) or median (interquartile range), where appropriate. Group 1: no infection; Group 2: local infection without blood stream infection; Group3: blood stream infection. Abbreviations: CRP = C- reactive protein; ICIS = intensive care infection score; PCT = procalcitonin; SIRS, systemic inflammatory response syndrome; WBC = white blood cells.

Table 3. Septic shock.

	No (n=280)	Yes (n=21)	**P**
Temperature (°C)	38.2 (1.5)	38.2 (1.6)	0.75
Heart rate (beats/ min)	108 (33)	119 (59)	0.04
Respiratory rate (breaths/ min)	28 (15)	29 (13)	0.85
WBC day 0 (10^9/L)	12.8 (9.5)	12.5 (17.6)	0.68
WBC day 1 (10^9/L)	12.4 (9.5)	14.1 (20.4)	0.60
WBC day 2 (10^9/L)	12.5 (9.4)	14.6 (14.4)	0.41
CRP day 0 (mg/L)	107 (144)	234 (182)	<0.001
CRP day 1 (mg/L)	124 (147)	327 (160)	<0.001
CRP day 2 (mg/L)	107 (144)	244 (205)	<0.001
PCT day 0 (μg/L)	1.15 (6.1)	32.2 (94.0)	<0.001
ICIS day 0	4 (5)	9 (6)	<0.001
ICIS day 1	4 (4)	8 (7)	<0.001
ICIS day 2	5 (5)	7 (6)	0.03

Median (interquartile range). Abbreviations: CRP = C- reactive protein; ICIS = intensive care infection score; PCT = procalcitonin; WBC = white blood cells.

Table 4. Receiver operating characteristic curve analysis to determine the optimum cut-off value of the different biomarkers at Day 0 for the prediction of infection (Group 2 + 3).

	Parameters						
Biomarkers	AUC (95% CI)	P	Cut-off	Sensitivity	Specificity	PPV	NPV
WBC (10^9/L)	0.53 (0.46, 0.60)	0.38	12.9	0.54	0.54	0.55	0.54
CRP (mg/L)	0.70 (0.64, 0.76)	<0.001	111	0.65	0.64	0.65	0.64
PCT (μg/L)	0.71 (0.66, 0.77)	<0.001	1.41	0.65	0.66	0.66	0.65
ICIS	0.73 (0.67, 0.79)	<0.001	5	0.66	0.71	0.70	0.67

Abbreviations: CI = confidence interval; CRP = C- reactive protein; ICIS = intensive care infection score; PCT = procalcitonin; PPV = positive predictive value; NPV = negative predictive value; WBC = white blood cells. The AUC for ICIS differed from that of WBC (P<0.001).

Discussion

This study evaluated the predictive values of ICIS, to discriminate between non-infectious systemic inflammation and infection (mostly sepsis) in critically ill patients with a suspicion of infection. The data suggest that ICIS is an useful marker to predict probable or proven infection and its severity and is non-inferior in this respect to CRP and PCT.

In the current study the frequency of probable or proven infection was 56% of patients when an infection was suspected, which is comparable with the reported frequency of 51-58% in a large studies on the epidemiology of sepsis in

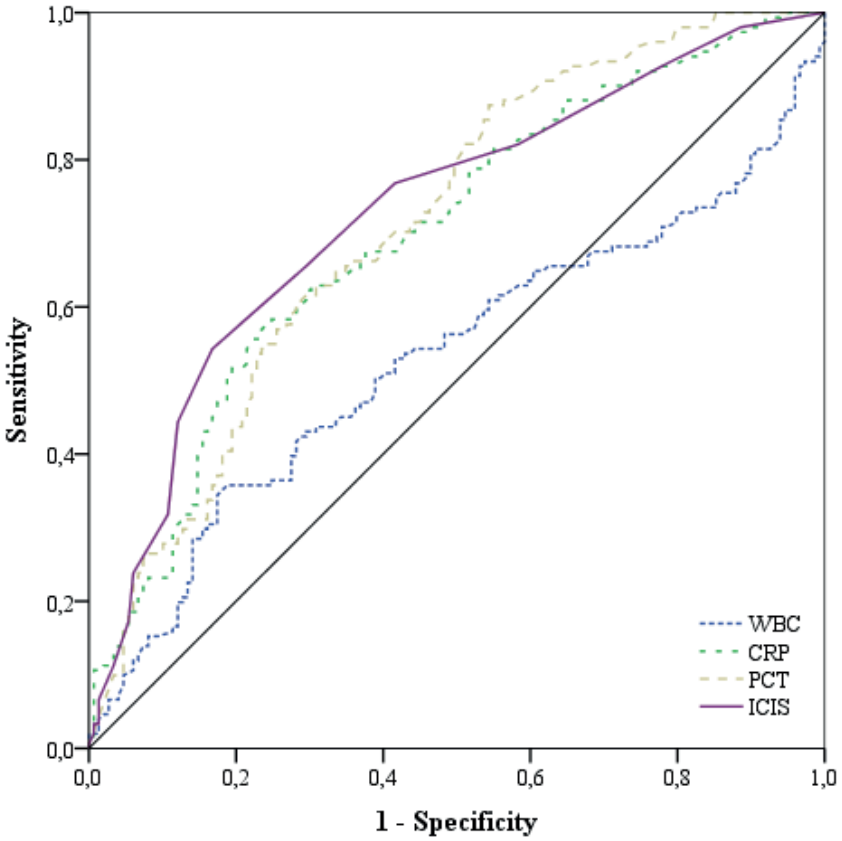


Figure 1. Area under the receiver operating characteristic (AUROC) curve of the four biomarkers for the prediction of infection during suspected infection episode I: for white blood cell count (WBC) 0.53, for C-reactive protein (CRP 0.70), for procalcitonin (PCT) 0.71 and for intensive care infection score (ICIS) 0.73.

the ICU [23]. The lung and abdomen were the most common origin of sepsis, followed by infections of soft tissues, as described before [24]. A large recently performed study showed that Gram-negative bacteria were isolated in 62% of patients with sepsis who had positive cultures, Gram-positive bacteria in 47%, and fungi in 19% [23]. The results are in contrast with our study, which suggests that Gram-positive isolates are most likely to cause infection. The difference can be explained by the fact that we use SDD in our ICU's, which is known to eliminate Gram-negative bacteria and fungi from the digestive tract [25]. Blood cultures are typically positive in approximately one third of the patients with sepsis, in line with the incidence of 20% in this study [24]. The overall ICU and hospital mortality rates were 28 and 37%, respectively. The results are compa-

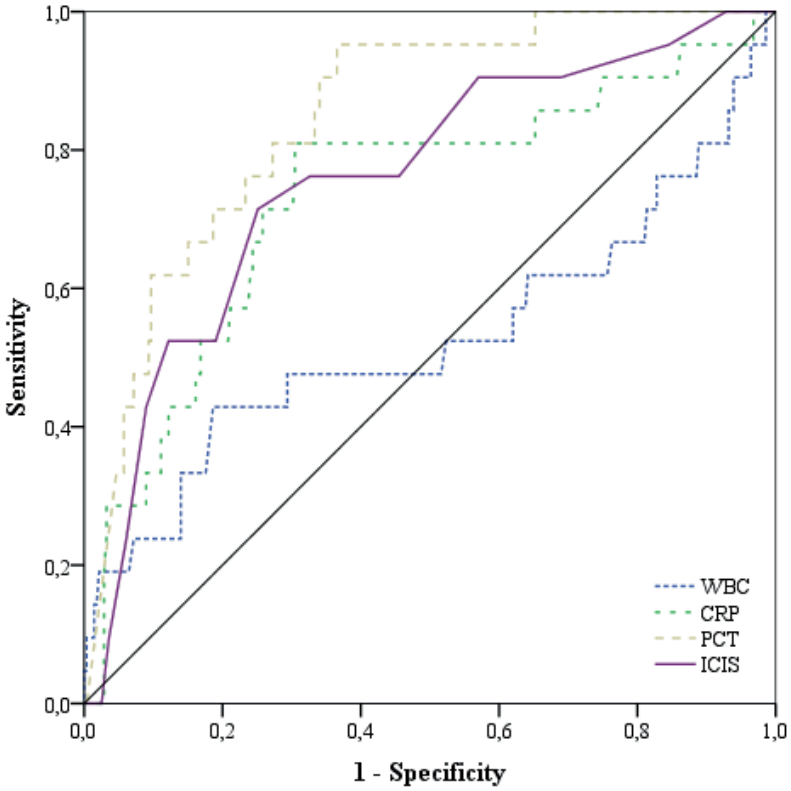


Figure 2. Area under the receiver operating characteristic (AUROC) curve of the four biomarkers for the prediction of septic shock during episode I: for white blood cell count (WBC) 0.53, for C-reactive protein (CRP) 0.73, for procalcitonin (PCT) 0.85 and for intensive care infection score (ICIS) 0.76.

rable with the reported rates in a European multi-center study of critically ill patients with sepsis [26].

In the current study the predictive values of WBC and CRP for infection and sepsis are comparable with previous studies which reported a low AUROC of 0.55-0.66 for WBC (sensitivity 65-91%; specificity 35-54%) and an intermediate AUROC of 0.64-0.77 for CRP (sensitivity 82-100%; specificity 40-64%) [4,5,10,11]. Large reviews reported an AUROC of 0.78-0.81 for PCT (sensitivity 42-100%; specificity 48-100%), comparable to our study [7,9]. The reported predictive value of ICIS in this study is lower compared with two previous studies, which reported an AUROC of 0.79 (sensitivity 70%; specificity 79%) and 0.85 (sensitivity 80%; specificity 75%), respectively [12,13]. Both studies investigated a relatively small number of patients or investigated postoperative critically ill patients only [12,13]. They were pilot studies to define the cut-off values of ICIS

as a marker of infection in critically ill patients and recommended determination of the suitability and effectiveness of this score in a prospective trial [12,13].

Using ICIS has several advantages over using CRP or PCT. First, no extra blood needs to be taken since the ICIS can be measured from the same K3EDTA tube which is used for the WBC measurement, thereby allowing routine daily measurements. Second, lower costs are involved because the ICIS measurement is performed on the same machine used for a WBC measurement. The major limitation is that in our study the predictive values of biomarkers including ICIS was not very high. Nevertheless, a high ICIS increases the likelihood of infection when suspected and a low ICIS decreases it. This may help the clinician in ordering extra test or start empiric antibiotics. The predictive value of ICIS for infection and septic shock is comparable with that of immature granulocytes percentage in a relatively small study in critically ill patients [27]. Both the immature granulocytes percentage and ICIS can be obtained routinely without extra blood sampling nor costs, though the current study focused on the diagnostic accuracy of ICIS and not its feasibility or cost-effectiveness. For future use the ICIS is expected to prove more robust.

In conclusion, the present study suggest that ICIS is a novel and potentially useful predictor of infection and sepsis in critically ill patients with a suspected infection. The ICIS score can be collected routinely without extra blood sampling and lower costs, yielding results within 15 minutes.

References

1. Circiumaru B, Baldock G, Cohen J. A prospective study of fever in the intensive care unit. Intensive Care Med 1999; 25: 668-673
2. Peres Bota D, Lopes Ferreira F, Mélot C, Vincent JL. Body temperature alterations in the critically ill. Intensive Care Med 2004; 30: 811-816
3. Vincent JL, Beumier M. Diagnostic and prognostic markers in sepsis. Expert Rev Anti Infect Ther 2013; 11: 265-275
4. Luzzani A, Polati E, Dorizzi R, Rungatscher A, Pavan R, Merlini A. Comparison of procalcitonin and C-reactive protein as markers of sepsis. Crit Care Med 2003; 31: 1737-1741
5. Charles PE, Kus E, AHO S, Prrin S, Doise J-M, Olsson N-O, Blettery B, Quenot J-P. Serum procalcitonin for the early recognition of nosocomial infection in the critically patients: a preliminary report. BMC Infect Dis 2009; 9: 49
6. Cid J, Garcia-Pardo G, Aguinaco R, Sánchez R, LIorente A. Neutrophil CD64: diagnostic accuracy and prognostic value in patients presenting to the emergency department. Eur J Clin Microbiol Infect Dis 2011; 30: 845-852
7. Kibe S, Adams K, Borlow G. Diagnostic and prognostic biomarkers of sepsis in critical care. J Antimicrob Chemother 2011; 66: i33-ii40
8. Vincent JL, Donadello K, Schmit X. Biomarkers in the critically ill patient: C-reactive protein. Crit Care Clin 2011; 27: 241-251
9. Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect Dis 2013; 13: 426-435
10. Bloos F, Reinhart K. Rapid diagnosis of sepsis. Virulence 2014; 5: 154-160
11. Garnacho-Montero J, Huici-Moreno MJ, Gutiérrer-Pizarraya A, López I, Márquez-Vacaro JA, Macher H, et al. Prognostic and diagnostic value of eosinopenia, C-reactive protein, procalcitonin and circulating cell-free DNA in critically ill patients admitted with suspicion of sepsis. Crit Care 2014; 18: R116
12. Nierhaus A, Linssen J, Wichmann D, Braune S, Kluge S. Use of a weighted, automated analysis of the differential blood count to differentiate sepsis from non-infectious systemic inflammation: The Intensive Care Infection Score (ICIS). Inflamm Allergy Drug Targets 2012; 11: 109-115
13. Weimann K, Zimmermann M, Spies CD, Wernecke KD, Vicherek O, Nachtigall I, et al. Intensive Care Infection Score – A new approach to distinguish between infectious and noninfectious processes in intensive care and medicosurgical patients. J Int Med Res 2015; 43: 435-451
14. Tanaka C, Nagai T, Nakamura M, Yamauchi Y, Noguchi K, Takimoto Y, et al. Automated hematology analyzer XE-5000 – Overview and basic performance. Sysmex J Int 2007; 17: 1-6
15. Smith JA. Neutrophils, host defense, and inflammation: a double-edged sword. J Leukoc Biol 1994; 56: 672-686
16. Franck S, Linssen J, Messinger M, Thomas L. Potential utility of Ret-Y in the diagnosis of iron- restricted erythropoiesis. Clin Chem 2004; 50: 1240-1242
17. Linssen J, Jennissen V, Hildmann J, Reisinger E, Schindler J, Malchau G, et al. Identification and quantification of high fluorescence-stained lymphocytes as antibody synthesizing/ secreting cells using the automated routine hematology analyzer XE-2100. Cytometry B Clin Cytom 2007; 72: 157-166
18. Bender L, Thaarup J, Varming K, Krarup H, Ellermann- Eriksen S, Ebbesen F. Early and late markers for the detection of early- onset neonatal sepsis. Dan Med Bull 2008; 55: 219-223
19. Theurl I, Aigner E, Theurl M, Nairz M, Seifert M, Schroll A, et al. Regulation of iron homeostasis in anemia of chronic disease and iron deficiency anemia: diagnostic and therapeutic implications. Blood 2009; 113: 5277-5286

20. Calandra T, Cohen J. International sepsis forum definition of infection in the ICU consensus conference. Crit Care Med 2005; 33: 1538-1548
21. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. Am J Infect Control 1988; 16: 128-140
22. Tape TG, Interpreting diagnostic tests, http://gim.unmc.edu/dxtests/, accessed 14 April 2016
23. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. J Am Med Ass 2009; 302: 2323-2329
24. Angus DC, Van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013; 369: 840-851
25. Bonten MJ, Kullberg BJ, Van Dalen R, Girbes AR, Hoepelman IM, Hustinx W, et al. Selective digestive decontamination in patients in intensive care. The Dutch working group on antibiotic policy. J Antimicrob Chemother 2000; 46: 351-362
26. Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, et al. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006; 34: 344-353
27. Van der Geest PJ, Mohseni M, Brouwer R, van der Hoven B, Steyerberg EW, Groeneveld AB. Immature granulocytes predict microbial infection and its adverse sequelae in the intensive care unit. J Crit Care 2014; 29: 523-527

Chapter 4

The diagnostic accuracy of procalcitonin for bacteraemia: a systematic review and meta-analysis

Sandra H. Hoeboer[1], Patrick J. van der Geest[1], Daan Nieboer[2] and A.B. Johan Groeneveld[1]

[*]Authors SHH and PJG contributed equally to this study.

[1] Department of Intensive Care Medicine, Erasmus Medical Center, Rotterdam, The Netherlands.
[2] Department of Public Health, Erasmus Medical Center, Rotterdam, The Netherlands.

Clinical Microbiology and infection 2015; 21: 474-81

Abstract

Objective

The diagnostic use of procalcitonin for bacterial infections remains a matter of debate. So far most studies used ambiguous outcome measures such as sepsis instead of infection. We performed a systematic review and meta-analysis to investigate the diagnostic accuracy of procalcitonin for bacteraemia, a proven bloodstream infection.

Methods

We searched all major databases from inception to June 2014 for original, English written, research articles that studied the diagnostic accuracy between procalcitonin and positive blood cultures in adult patients. We calculated the area under the summary receiver-operating characteristic curves (SROC) and pooled sensitivities and specificities. To minimise potential heterogeneity we performed subgroup analyses.

Results

In total 58 of 1,567 eligible studies were included in the meta-analysis and provided a total of 16,514 patients of whom 3,420 suffered from bacteraemia. In the overall analysis the SROC was 0.79. The optimal and most widely used procalcitonin cut-off value was 0.5 ng/mL with a corresponding sensitivity of 76% and specificity of 69%. In subgroup analyses the lowest SROC was found in immunocompromised/neutropenic patients (0.71), the highest SROC was found in intensive care patients (0.88), sensitivities ranging 66-89% and specificities 55-78%.

Conclusions

In spite of study heterogeneity, procalcitonin had a fair diagnostic accuracy for bacteraemia in adult patients suspected of infection or sepsis. In particular low procalcitonin levels can be used to rule out the presence of bacteraemia. Further research on the safety and efficacy of procalcitonin as a single diagnostic tool to withhold taking blood cultures is needed.

Introduction

Infection and the subsequent sepsis syndrome are associated with morbidity and mortality [1,2]. The fear of undertreatment leads to the routine collection of specimen for microbiological culture and initiation of empiric antibiotic therapy [3]. On the other hand, antibiotic overuse increases microbial selection and resistance and can cause adverse drug reactions [4]. To assist the diagnosis of infection in clinical practice its symptoms have been grouped into the systemic inflammatory response syndrome (SIRS [5]. A clinically suspected or proven infection in the presence of SIRS is termed sepsis [5]. In recent years authors have studied the use of biomarkers, like procalcitonin, to improve the diagnosis of the sepsis syndrome rather than of proven infection [6-9]. The use of the sepsis syndrome as a surrogate for proven infection as an outcome parameter may be too sensitive and nonspecific. This could partially explain the contradicting results in previous studies [6-9,10] and meta-analyses on the diagnostic use of procalcitonin for sepsis [11-18].

The definition of proven local infection remains matter of debate and we therefore study the more robustly defined proven bloodstream infections, i.e. bacteraemia. Bacteraemia can be identified in about 30% of septic patients and necessitates further diagnostic evaluation [19]. However, culture results take several days and can be falsely negative in patients on antibiotic treatment [20-22]. Recent studies demonstrated that procalcitonin can accurately predict bacteraemia in patients with community-acquired pneumonia [23], acute fever [24], and in elderly patients suspected of infection [25]. Procalcitonin can also accurately discriminate between true bacteraemia and coagulase negative staphylococci-contaminated blood cultures [26]. Another study demonstrated that bacteraemia is unlikely when procalcitonin levels are low [27]. Some meta-analyses focused on the diagnostic value of procalcitonin for microbiologically confirmed local infection [28-39] or bacteraemia [40]. However, the number of included studies was small, specific patient subgroups were analysed or studies concerning sepsis were included as well [28-40].

We therefore performed a systematic review and meta-analysis to investigate the diagnostic accuracy of procalcitonin for bacteraemia. Our hypothesis is that in adult patients suspected for infection or sepsis procalcitonin is a useful biomarker of bacteraemia.

Methods

Search strategy and study selection

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for reporting this systematic review and meta-analysis [41]. A flowchart of the literature search can be found in Figure 1. All prospective and retrospective, original, observational (case-control, cross sectional, cohort and longitudinal) studies published in English from inception until June 2014 were considered eligible for inclusion. Studies were screened by title and abstract and definite inclusion was decided upon after full text review.

We included studies on adult hospitalised patients suspected of infection or sepsis, in which bacteraemia with a known pathogen was confirmed by blood culture and measurement of procalcitonin levels was performed within 24 hours of inclusion. Studies had to give a detailed description of patient groups and demographic variables. The comparison of procalcitonin levels had to be between hospitalised patients with and without bacteraemia, regardless of clinical symptoms. To be included for analysis studies had to report the diagnostic accuracy estimates of procalcitonin for bacteraemia; knowingly area under the curve (AUC), sensitivity, specificity and corresponding P-values. The corresponding authors of eligible studies that did not provide sufficient data for meta-analysis were contacted to retrieve additional data. We excluded case-control studies were controls were healthy subjects, reviews, meta-analyses, case reports, editorials, commentaries, letters, meeting abstracts, poster presentations, animal studies and research performed in children (<18 years old). Two investigators (SHH and PJG) independently evaluated all eligible studies for inclusion and extracted the data. In case of disagreement a third investigator (ABJG) was consulted.

Quality assessment

We used the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool [42], scores range from 0 to 14, to assess the methodological quality of included studies.

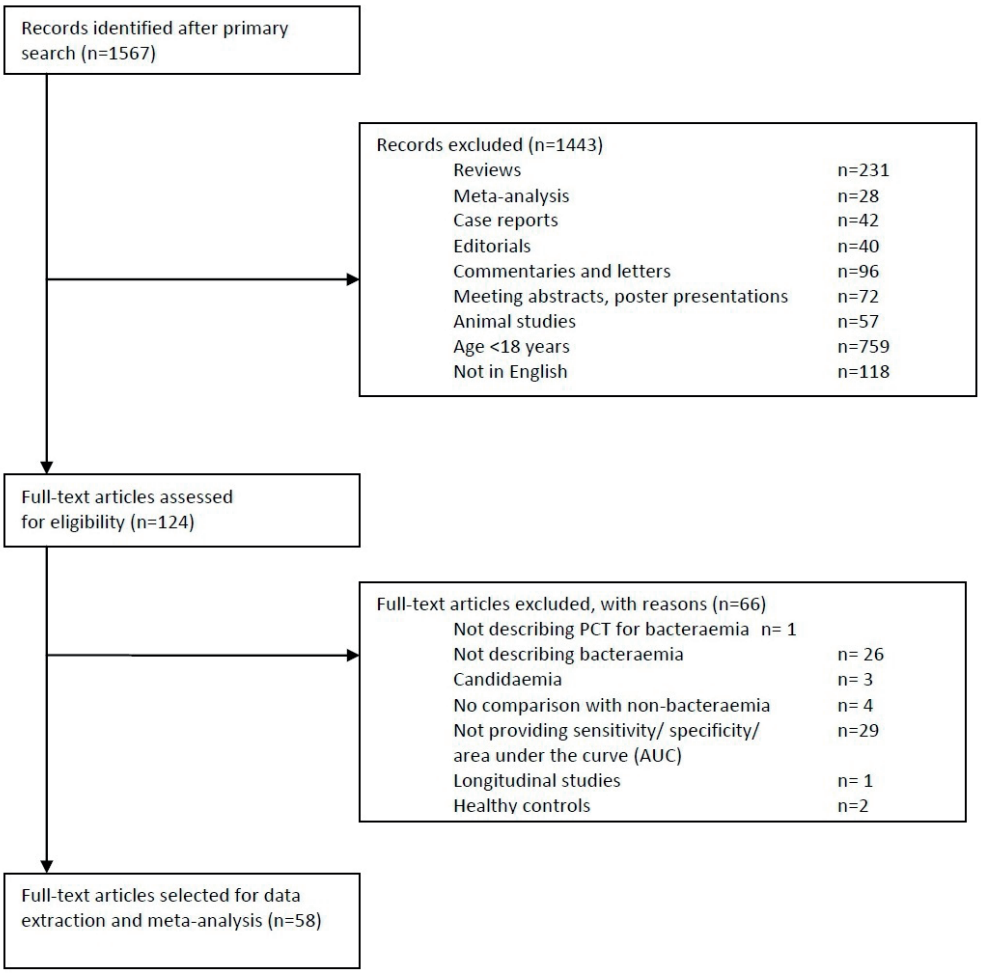


Figure 1. Flow chart of literature search. PCT = procalcitonin.
Search databases: PubMed, Medline, Embase, ISI Web of Knowledge, the Cochrane Library, Scopus, BioMed Central, and Science Direct.
Search strategy: (procalcitonin OR PCT) AND (bacterial infection OR bacteraemia OR bloodstream infection).

Statistical methods

To avoid double inclusion of the same patient group we only included one sensitivity and specificity from each article, unless results clearly came from different patient groups. We used the bivariate random-effects regression model for pooling the sensitivity and specificity estimates, as recommended by the Cochrane Diagnostic Test Accuracy Working Group [43]. The bivariate model takes into account the potential trade-off between sensitivity and specificity by explicitly

incorporating this negative correlation in the analysis [44,45]. Cut-off values differed among the included studies, the cut-off value closest to 0.5 ng/mL was used for the analysis if multiple cut-off values were given. The 0.5 ng/ml cut-off was chosen based on recommendations of the manufacturer, current literature [46-49] and was the cut-off used most often in the included studies (Table 1). Summary receiver-operating characteristics curves (SROC) were drawn using the bivariate model. The closer the curve is to the upper left-hand corner of the SROC curve plot, the better the overall accuracy of the test. An area under the SROC curve between 0.90-1.0 is considered as excellent diagnostic accuracy, 0.80-0.90 as good, 0.70-0.80 as fair, 0.60-0.70 as poor and 0.50-0.60 as fail [50]. We expected substantial heterogeneity in the of the overall analysis and in order to obtain more homogenous results subgroup analysis were performed. First, we calculated the diagnostic accuracy in specific patient subgroups based on their underlying disease. We calculated the diagnostic accuracy in studies comparing bacteraemia vs. non-bacteraemia in patients with SIRS and comparing bacteraemia vs. non-bacteraemia in patients with SIRS developing localised infections. When a specific subgroup for the controls could not be identified we categorised the study in the category non-bacteraemia. We studied the diagnostic accuracy of procalcitonin for bacteraemia in immunocompromised/neutropenic and immunocompetent patients separately. We categorised all studies according to department of inclusion. Finally, we also studied retrospective studies separately from prospective studies. We tested for a threshold effect by adding a covariate for threshold to the bivariate model.

We used IBM statistics 21.0 (IBM SPSS, Chicago, IL, USA) and R 3.1.1 (Vienna, Austria XX) to analyse the data. The R package mada was used to perform the pooling of sensitivity and specificity and generating of SROC-curves. Pooled sensitivity and specificity estimates were generated, with their 95% confidence interval (CI). To assess heterogeneity among studies I^2 and X^2/cochrane Q statistics were performed. We used the Deeks funnel plot asymmetry test to evaluate potential publication bias [51]. $P<0.10$ for the slope coefficient is considered as significant asymmetry, which indicates potential publication bias. All other tests were two-sided and a $P<0.05$ was considered statistically significant; exact P-values >0.001 are given.

Table 1. Study characteristics.

Reference	Country	Inclusion criteria	Study population (N)	With bacteraemia (N)	Male (%)	Age (years)	Department	Type of patients	Immunocompromised	Assay type	Cut-off values, ng/mL	Quadas
Aalto 2004 [58]	Finland	Suspicion of systemic infection	92	13	48	52	ED	medical	no	1	0.4	12
Albrich 2011 [59]	Switzerland	Performing blood cultures[R]	295	16	-	48	ED	medical	no	2	0.15	12
Bell 2003[60]	Australia	SIRS and suspicion of infection	123	12	66	61	ICU	mixed	no	1	3.03	11
Bogar 2006 [61]	Hungary	New onset fever[*]	39	23	71	56	ICU	mixed	yes	1	N.A.	12
Bossink 1999 [62]	Netherlands	New onset fever	300	53	51	60	ward	medical	no	1	N.A.	12
Caterino 2004 [63]	USA	>65 years and performing blood cultures[*]	108	14	50	76	ED	medical	no	1	0.5	13
Charles 2008 [64]	France	Critically ill with BSI and VAP[R]	161	117	58	65	ICU	mixed	no	2	0.5	13
Chen 2011 [65]	China	Suspicion of catheter-related BSI	55	25	65	53	mixed	medical	no	1	3.1	12
Cheval 2000 [66]	France	Sepsis/ septic shock and no infection	60	9	57	58	ward	mixed	no	1	0.55	10
Chirouze 2002 [67]	France	Acute fever	165	22	58	58	ward	medical	no	1	0.12	12
Dwolatzky 2005 [68]	Israel	Proven microbial infection	187	16	30	83	ED	medical	no	1	0.5	13
Engel 1999 [69]	Germany	Febrile neutropenia	44	15	-	47	ward	medical	yes	1	0.51	11
Gac 2011 [70]	France	Febrile neutropenia[*]	29	10	53	56	ward	medical	yes	3	0.5	11
Gaini 2007 [71]	Denmark	Suspicion of severe infection	154	34	50	61	ward	medical	no	2	2.19	13
Giamarellos 2001 [72]	Greece	Febrile neutropenia.	115	28	70	56	ward	medical	yes	1	0.5	11
Giamarellou 2004 [73]	Greece	Febrile neutropenia.	158	52	56	52	ward	medical	yes	2	1	11
Guinard-Barbier 2011 [53]	France	Acute pyelonephritis[*]	347	58	8	33	ED	medical	no	2	0.3	13
Ha 2013 [74]	South Korea	Acute pyelonephritis[R]	147	84	15	61	ED	medical	no	4	0.5	12
Hoeboer 2012 [75]	Netherlands	New onset fever	101	12	68	64	ICU	mixed	no	2	2.44	12
Hoenigl 2013 [54]	Austria	SIRS and suspicion of infection[*]	132	55	48	69	ED	medical	no	4	N.A.	13
Hoenigl 2014 [76]	Austria	SIRS and performing blood cultures[*]	898	666	58	67	ED	medical	no	3	0.5	12
Jeong 2012 [77]	South Korea	Suspicion of bacteraemia [R]	3343	331	59	65	mixed	medical	no	1	0.35	12

Table 1. Study characteristics. (continued)

Reference	Country	Inclusion criteria	Study population (N)	With bacteraemia (N)	Male (%)	Age (years)	Department	Type of patients	Immunocompromised	Assay type	Cut-off values, ng/mL	Quadas
Jimeno 2004 [78]	Spain	Febrile neutropenia.	104	15	38	58	ward	medical	yes	1	0.5	11
Kallio 2000 [79]	Finland	Cancer and suspicion for infection	56	8	63	57	ward	medical	yes	1	0.36	12
Karlsson 2010 [80]	Finland	Severe sepsis or septic shock	160	69	68	60	ICU	mixed	no	3	1.2	14
Kim D 2011 [81]	South Korea	Febrile neutropenia.	286	38	57	39	ED	medical	yes	-	0.5	7
Kim M 2011 [24]	South Korea	Fever and performing blood cultures	252	31	44	54	ED	medical	no	4	0.5	7
Koivula 2011 [82]	Finland	Febrile neutropenia.	90	21	66	56	ward	medical	yes	2	0.5	11
Lai 2010 [25]	Taiwan	SIRS and suspicion of infection	155	48	60	77	ED	medical	no	2	0.38	13
Lee 2013 [83]	South Korea	PCT measurements [R]	357	199	53	66	mixed	medical	no	4	0.55	13
Liaudat 2001[84]	Switzerland	Performing blood cultures	200	50	52	60	mixed	mixed	no	1	0.5	12
Loonen 2014 [85]	Netherlands	SIRS and suspicion of infection[R]	125	27	60	65	ED	medical	no	3	2.0	14
Mencacci 2012 [86]	Italy	Fever and suspicion of sepsis	1009	133	55	69	mixed	mixed	no	4	0.37	13
Menendez 2012 [55]	Spain	Pneumonia[*]	685	48	59	64	ward	medical	no	6	0.36	12
Muller 2010 [23]	Switzerland	Pneumonia[*]	925	73	59	73	ED	medical	no	2	0.5	13
Munoz 2004 [87]	Spain	Fever	103	23	31	59	ward	mixed	no	1	0.1	11
Nakamura 2009 [88]	Japan	High fever suspicion of bacteraemia	116	65	65	59	ICU	mixed	no	4	0.38	10
Nieuwkoop van 2010 [89]	Netherlands	Fever and urinary tract infection	581	131	38	66	mixed	mixed	no	2	0.5	13
Pereira 2013 [90]	Portugal	Pneumonia[*]	108	15	63	61	ICU	medical	no	4	17	12
Persson 2004 [91]	Sweden	Febrile neutropenia.	94	21	41	54	ward	medical	yes	6	0.5	11
Prat 2008 [92]	Spain	Febrile neutropenia.	61	19	51	47	ward	medical	yes	2	0.5	8
Ratzinger 2014 [93]	Austria	Suspicion of infection and performing blood cultures	298	75	58	58	ward	mixed	no	-	0.35	13
Riedel 2011 [27]	USA	Signs of infection and performing blood cultures[R]	367	19	-	48	ED	medical	no	2	0.15	14

Table 1. Study characteristics. (continued)

Reference	Country	Inclusion criteria	Study population (N)	With bacteraemia (N)	Male (%)	Age (years)	Department	Type of patients	Immunocompromised	Assay type	Cut-off values, ng/mL	Quadas
Rintala 2001 [94]	Finland	Fever and a proven microbial infection	29	13	52	49	mixed	medical	no	1	<0.5	11
Robinson 2011 [95]	Switzerland	Febrile neutropenia	194	33	61	57	ward	medical	yes	2	0.5	12
Romualdo 2014 [96]	Spain	SIRS and suspicion of infection	226	37	58	69	ED	medical	no	3	0.45	13
Schuetz 2007 [26]	Switzerland	Positive blood cultures	19	7	65	63	mixed	medical	no	5	0.1	12
Schuetz 2008 [97]	Switzerland	Pneumonia	281	34	62	74	ward	medical	no	2	1.34	13
Shi 2013 [98]	China	New onset fever	106	60	67	64	ICU	mixed	no	4	N.A.	12
Shomali 2012 [99]	USA	Cancer and new fever	248	30	57	56	ward	medical	no	2	0.5	13
Su 2011 [100]	Taiwan	Performing blood cultures*	558	84	57	61	ED	medical	no	1	0.5	12
Suarez-Santamaria 2010 [101]	Spain	Proven microbial infection	205	36	58	65	ED	mixed	no	6	N.A.	13
Theodorou 2012 [57]	Greece	Suspicion of catheter related BSI*	46	26	61	48	ICU	mixed	no	1	0.7	13
Tromp 2012 [102]	Netherlands	SIRS and suspicion of infection	342	55	56	59	ED	mixed	no	2	0.5	13
Tsalik 2012 [103]	USA	SIRS and suspicion of infection	336	55	52	52	ED	medical	no	3	0.5	12
Vanska 2012 [104]	Finland	Febrile neutropenia.	100	19	61	66	ward	medical	yes	3	0.13	10
von Lilienfeld-Toal 2004 [105]	Germany	Febrile neutropenia.	53	18	48	57	ward	medical	yes	1	0.62	12
Wang 2013 [10]	China	SIRS and performing blood cultures R*	586	120	65	54	mixed	mixed	no	4	0.5	12
Total			**16,514**	**3,420**								**12 (7-14)**

The mean or median age is provided, if mean/median was not provided, the mean age was manually calculated of the subgroups. Studies in which patients were excluded because of antibiotic use prior to PCT measurement are marked with an *. All studies have a prospective study design, retrospective studies are marked with R. Assay type: 1 = Lumitest Brahms, 2 = Kryptor Brahms, 3 = Elecsys Brahms Cobas Analyzer, 4 = Vidas Biomereux, 5= PCT sensitive Lia Brahms, 6 = Liason Brahms PCT. ED = emergency department; ICU = intensive care unit; USA = United States of America; QUADAS = quality assessment of diagnostic accuracy studies. BSI = bloodstream infection; SIRS = systemic inflammatory response syndrome; VAP = ventilator associated pneumonia.

Table 2. Excluded studies.

Reason of exclusion	Excluded study	
Not providing PCT	Pettila 2002 [6]	
Not studying bacteraemia	Adamzik 2010 [106]	Pizzolato 2014 [119]
	Barati 2008 [107]	Quiroga 2014 [120]
	Bele 2011[108]	Reynolds 2012 [121]
	Bugden 2004 [109]	Rowther 2009 [122]
	Delevaux 2002 [110]	Sakr 2008 [123]
	Fluri 2012 [111]	Stankovic 2010 [124]
	Freund 2012 [112]	Steichen 2009 [125]
	Hettwer 2010 [113]	Uusitalo 2011 [126]
	Jereb 2009 [114]	Viallon 2008 [127]
	van Langevelde 2000 [115]	Wang 2014 [1]
	Magrini 2013 [116]	Wunderink 2012 [128]
	Oberhoffer 2000 [117]	Yan 2014 [129]
	Patil 2012 [118]	Zhu 2014 [130]
Studying candidaemia	Charles 2006 [131]	Martini 2010 [133]
	Charles 2009 [132]	
No comparison to non-bacteraemia	Charles 2008 [134]	Mueller 2004 [136]
	Knudsen 2010 [135]	Shomali 2013 [137]
Not providing sensitivity or specificity	Ahn 2010 [138]	Lee 2014 [139]
No AUC values of PCT for bacteraemia	Al Shuaibi 2013 [140]	Lehmann 2010 [154]
	Aouifi 2000 [141]	Lodes 2012 [155]
	Bloos 2012 [142]	Mauro 2012 [156]
	Boussekey 2005 [143]	Park 2012 [56]
	Cuculi 2008 [144]	Peters 2006 [157]
	Endo 2012 [145]	Previsdomini 2012 [158]
	Feld 2008 [146]	Sandri 2008 [159]
	Foushee 2012 [147]	Scott 2003 [160]
	Gille johnson 2012 [148]	Su 2012 [161]
	Groeneveld 2008 [149]	Svaldi 2001 [162]
	Guven 2002 [150]	Ugarte 1999 [163]
	Juutilainen 2011 [151]	von Lilienfeld 2009 [164]
	Kim 2010 [152]	Yilmaz 2011 [165]
	Kruif de 2008 [153]	
Longitudinal studies not providing PCT on inclusion	Lavrentieva 2012 [166]	
Healthy controls	Gaini 2008 [167]	Kocazeybek [168]

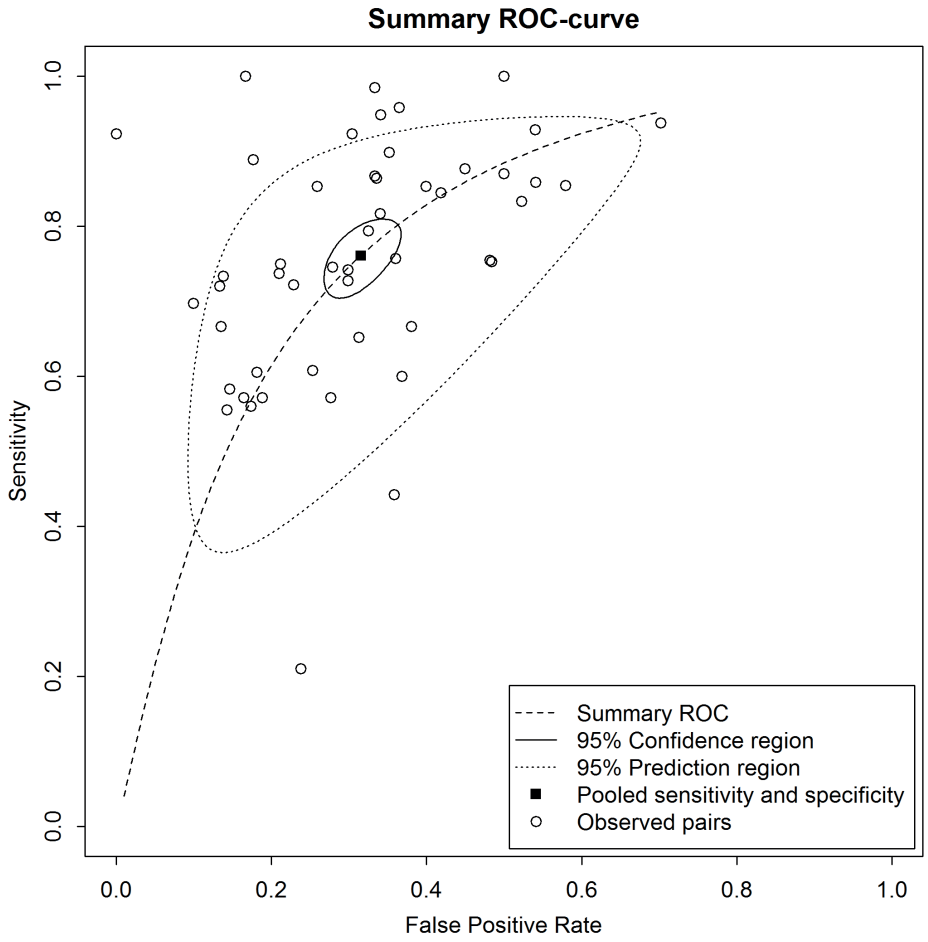


Figure 2. Summary receiver-operating characteristic (SROC) curve plot of procalcitonin for the diagnosis of bacteraemia, including all studies (n=58). Individual studies are shown as open circles. Summary point is shown as a closed square, representing sensitivity estimates pooled by using bivariate random-effects regression model. The area under the SROC curve (dashed line) is 0.79, pooled sensitivity 76% and specificity 69%. The 95% confidence region displays the 95% confidence interval of the pooled sensitivity and specificity. The 95% prediction region is the region for a forecast of the true sensitivity and specificity in a future study.

Results

Literature search

The literature search resulted in a total of 1,567 articles of which 1,443 studies were excluded because of: written language other than English (n=118), age <18 years (n=759), in vitro/animal studies (n=57) or lack of original data (reviews, meta-analysis, case reports, editorials, commentaries and letters, meeting

Table 3. Raw QUADAS scores.

QUADAS item Reference	1	2	3	4	5	6	7	8	9	10	11	12	13	14	QUADAS SCORE
Aalto 2004 [58]	y	y	y	y	y	y	y	y	y	y	u	y	n	y	12
Albrich 2011 [59]	y	y	y	y	y	y	y	y	y	y	n	y	n	y	12
Bell 2003 [60]	y	y	y	n	y	y	y	y	y	y	y	y	u	n	11
Bogar 2006 [61]	y	y	y	y	y	y	y	y	y	n	y	y	n	y	12
Bossink 1999 [62]	y	y	y	y	y	y	y	y	y	n	y	y	n	y	12
Caterino 2004 [63]	y	y	y	y	y	y	y	y	y	y	n	y	y	y	13
Charles 2008 [64]	y	n	y	y	y	y	y	y	y	y	y	y	y	y	13
Chen 2011 [65]	y	y	y	y	y	y	y	y	y	y	y	y	u	u	12
Cheval 2000 [66]	y	n	y	y	y	y	y	y	n	y	y	y	u	u	10
Chirouze 2002 [67]	n	y	y	y	y	y	y	y	y	y	u	y	y	y	12
Dwolatzky 2005 [68]	y	n	y	y	y	y	y	y	y	y	y	y	y	y	13
Engel 1999 [69]	y	y	y	y	y	y	y	y	n	y	u	y	y	n	11
Gac 2011 [70]	y	n	y	y	y	y	y	y	n	u	y	y	y	y	11
Gaini 2007 [71]	y	y	y	y	y	y	y	y	y	y	n	y	y	y	13
Giamarellos 2001 [72]	y	y	y	y	n	n	y	y	y	y	n	y	y	y	11
Giamarellou 2004 [73]	y	n	y	y	y	y	y	y	n	y	y	y	y	n	11
Guinard-Barbier 2001 [53]	y	y	y	y	y	y	y	y	y	u	y	y	y	y	13
Ha 2013 [74]	y	n	y	y	y	y	y	y	y	y	y	y	y	n	12
Hoeboer 2012 [75]	y	y	y	y	y	y	y	y	y	n	y	y	n	y	12
Hoenigl 2013 [54]	y	y	y	y	y	y	y	y	y	u	y	y	y	y	13
Hoenigl 2014 [76]	y	y	y	y	y	y	y	y	y	y	y	y	n	n	12
Jeong 2012 [77]	y	n	y	y	y	y	y	y	y	y	y	y	y	n	12
Jimeno 2004 [78]	y	y	y	n	n	y	y	y	y	y	n	y	y	y	11
Kallio 2000 [79]	y	n	y	y	y	y	y	y	n	y	y	y	y	y	12
Karlsson 2010 [80]	y	y	y	y	y	y	y	y	y	y	y	y	y	y	14
Kim D 2011 [81]	y	n	y	y	y	y	y	n	n	u	u	y	u	n	7
Kim M 2011 [24]	y	n	y	y	y	y	y	u	u	u	u	y	u	u	7
Koivula 2011 [82]	y	n	y	y	y	y	y	y	y	n	n	y	y	y	11
Lai 2010 [25]	y	y	y	y	y	y	y	y	y	y	y	y	y	n	13
Lee 2013 [83]	y	y	y	y	y	y	y	y	y	y	y	y	n	y	13
Liaudat 2001 [84]	y	u	y	y	y	y	y	y	y	y	n	y	y	y	12
Loonen 2014 [85]	y	y	y	y	y	y	y	y	y	y	y	y	y	y	14
Mencacci 2012 [86]	y	y	y	y	y	y	y	y	y	y	y	y	y	n	13
Menendez 2012 [55]	y	y	y	y	y	y	y	y	y	y	y	y	n	n	12
Muller 2010 [23]	y	y	y	y	y	y	y	y	y	y	y	y	n	y	13
Munoz 2004 [87]	y	y	y	n	y	y	y	y	y	y	y	y	u	u	11
Nakamura 2009 [88]	y	n	y	y	y	y	y	n	n	y	y	y	y	n	10
Nieuwkoop van 2010 [89]	y	y	y	y	y	y	y	y	y	y	y	y	n	y	13

Table 3. Raw QUADAS scores. (continued)

QUADAS item Reference	1	2	3	4	5	6	7	8	9	10	11	12	13	14	QUADAS SCORE
Pereira 2013 [90]	y	y	y	y	y	y	y	y	y	u	y	y	y	n	12
Persson 2004 [91]	y	n	y	y	y	y	y	y	y	n	y	y	y	n	11
Prat 2008 [92]	y	u	y	y	y	y	y	y	u	n	u	y	u	u	8
Ratzinger 2014 [93]	y	y	y	y	y	y	y	y	y	y	y	y	u	y	13
Riedel 2011 [27]	y	y	y	y	y	y	y	y	y	y	y	y	y	y	14
Rintala 2001 [94]	y	n	y	y	y	y	y	y	y	y	y	y	u	n	11
Robinson 2011 [95]	y	y	y	y	y	y	y	y	y	n	y	y	y	n	12
Romualdo 2014 [96]	y	y	y	y	y	y	y	y	y	y	y	y	n	y	13
Schuetz 2007 [26]	y	y	y	y	y	y	y	y	n	y	y	y	y	n	12
Schuetz 2008 [97]	y	y	y	y	y	y	y	y	y	y	y	y	y	n	13
Shi 2013 [98]	y	y	y	y	y	y	y	y	y	n	y	y	y	n	12
Shomali 2012 [99]	y	y	y	y	y	y	y	y	y	y	y	y	y	n	13
Su 2011 [100]	y	y	y	y	y	y	y	y	y	y	n	y	y	n	12
Suarez-Santamaria 2010 [101]	y	y	y	y	y	y	y	y	y	y	y	y	y	n	13
Theodorou 2012 [57]	y	y	y	y	y	y	y	y	y	y	y	y	n	y	13
Tromp 2012 [102]	y	y	y	y	y	y	y	y	y	y	y	y	n	y	13
Tsalik 2012 [103]	y	y	y	y	y	y	y	y	y	u	u	y	y	y	12
Vanska 2012 [104]	y	y	y	y	y	y	y	y	y	u	u	y	u	u	10
Von Lillienfeld-Toal 2004 [105]	y	y	y	y	y	y	y	y	n	y	y	y	y	n	12
Wang 2013 [10]	y	n	y	y	y	y	y	y	y	y	y	y	y	n	12

The quality assessment of studies of diagnostic accuracy checklist. Item 1: Was the spectrum of patients representative of the patients who will receive the test in practice?; 2: Were selection criteria clearly described?; 3: Is the reference standard likely to correctly classify the target condition?; 4: Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?; 5:Did the whole study population or a random selection of the sample, receive verification using a reference standard for diagnosis?; 6: Did patients receive the same reference standard regardless of the index test result?; 7: Was the reference standard independent of the index test?; 8: Was the execution of the index test described in sufficient detail to permit replication of the test?; 9: Was the execution of the reference standard described in sufficient detail to permit its replication?; 10: Were the index test results interpreted without the knowledge of the results of the reference standard?; 11: Were the reference standard results interpreted without knowledge of the index test results?; 12: Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?; 13: Were uninterpretable / intermediate test results reported?; 14: Were withdrawals from the study explained? Each item can be answered with yes (Y), no (N) or unknown (U).

abstract, poster presentations, n=509). We performed a full text review of the 124 articles considered eligible for inclusion, which resulted in the exclusion of another 66 studies whom did not provide AUC values/ sensitivity/ specificity (n=29), did not study bacteraemia (n=26), did not compare to non-bacteraemia (n=4), studied candidemia (n=3), used healthy controls (n=2), did not provide

the procalcitonin level for bacteraemia (n=1), or used a longitudinal study design and analysis (n=1). The remaining 58 articles were used in the meta-analysis. Table 2 depicts the 66 studies excluded after full text review.

Study characteristics and quality assessment

Table 1 provides some details of the included studies. In total, 16,514 patients of whom 3,420 suffered from bacteraemia were included. There was a slight tendency towards male preponderance. The average age ranged from 33 to 77 years. Eight studies had a retrospective and 50 a prospective study design. All 58 studies provided AUC values, but only 49 studies provided sensitivity and specificity. The cut-off values varied between 0.10 and 17 ng/mL. All samples for blood culture and procalcitonin measurement were collected on inclusion or

Table 4. Accuracy estimates.

		Pooled sensitivity	Pooled specificity	Heterogeneity (%)		
Analysis	**AUC**	**(95% CI)**	**(95% CI)**	**I^2**	**X^2/ Q**	***p***
Overall (N=3,420)	0.79	76 (72-80)	69 (64-72)	86%	1397	<0.001
Control group						
Non-bacteraemia (N=1,884)	0.78	72 (66-78)	74 (69-76)	88%	1070	<0.001
SIRS (N=931)	0.78	76 (60-87)	66 (44-82)	83%	114	<0.001
Local infection and/or sepsis (N=605)	0.84	84 (80-87)	55 (47-63)	71%	162	<0.001
Immunocompromised/ neutropenic						
Yes (N=320)	0.71	66 (54-76)	78 (71-83)	76%	120	<0.001
No (N=3,100)	0.79	79 (75-83)	65 (60-65)	81%	926	<0.001
Department						
ICU (N=399)	0.88	89 (79-94)	68 (57-77)	77%	54	<0.001
Mixed (N=1,009)	0.77	76 (65-85)	66 (57-76)	31%	501	<0.001
Ward (N=587)	0.76	71 (63-78)	71 (64-77)	90%	433	<0.001
ED (N=1,425)	0.78	76 (69-82)	68 (61-75)	77%	285	<0.001
Study type						
Prospective (N=2,507)	0.79	76 (71-80)	69 (64-73)	86%	721	<0.001
Retrospective (N=913)	0.79	78 (66-86)	68 (56-78)	79%	636	<0.001

X2/Q = X2/cochrane Q, CI = confidence interval; ED = emergency department; ICU = intensive care unit; mixed = ICU/ ED/ ward together; SIRS = systemic inflammatory response syndrome.

within 24 hours at the emergency department, ward and/or intensive care unit. The median QUADAS score was 12 (range 7-14) the per item QUADAS scores are presented in Table 3. Problematic QUADAS items were: the description of selection criteria and description of the execution of the reference standard, whether the index test results were interpreted without knowledge of the results of the reference standard and vice versa, reporting of uninterpretable/intermediate test results and the explanation of withdrawals.

The diagnostic accuracy of procalcitonin for bacteraemia

In the overall analysis the area under the SROC was 0.79 (Figure 2 and Table 4). The optimal and most widely used procalcitonin cut-off value was 0.5 ng/mL (Table 5) and corresponded with a 76% sensitivity and 69% specificity (Table 4). In Figure 3, the sensitivity and specificity per study are given. The lowest SROC was found in immunocompromised/neutropenic patients (0.71), the highest

Table 5. Accuracy estimates for different cut-off values.

		Pooled sensitivity	Pooled specificity	Heterogeneity (%)		
Analysis	**AUC**	**(95% CI)**	**(95% CI)**	**I^2**	**X^2/ Q**	***p***
Cut-off 0.1	0.73	91 (82-96)	35 (22-51)	88%	385	<0.001
Cut-off 0.5	0.77	74 (66-81)	68 (61-75)	75%	478	<0.001
Cut-off 1.0	0.76	67 (52-78)	74 (67-80)	85%	154	<0.001
Cut-off 2.0	0.63	50 (31-69)	83 (64-94)	92%	313	<0.001

X2/Q = X2/cochrane Q, CI = confidence interval.

Table 6. 2x2 tables with corresponding sensitivity, specificity, PPV and NPV.

	Ward			Emergency Department			Intensive Care Unit		
	BSI+	BSI-		BSI+	BSI-		BSI+	BSI-	
PCT+	7	26	33	6	29	35	11	28	39
PCT-	3	64	67	2	63	65	1	60	61
	10	90	100	8	92	100	12	88	100
Prevalence			10%			8%			12%
Sensitivity			71%			76%			89%
Specificity			71%			68%			68%
PPV			21%			17%			28%
NPV			95%			97%			98%

BSI = blood stream infection; NPV = negative predictive value; PCT = procalcitonin; PPV = positive predictive value, += positive test, -= negative test.

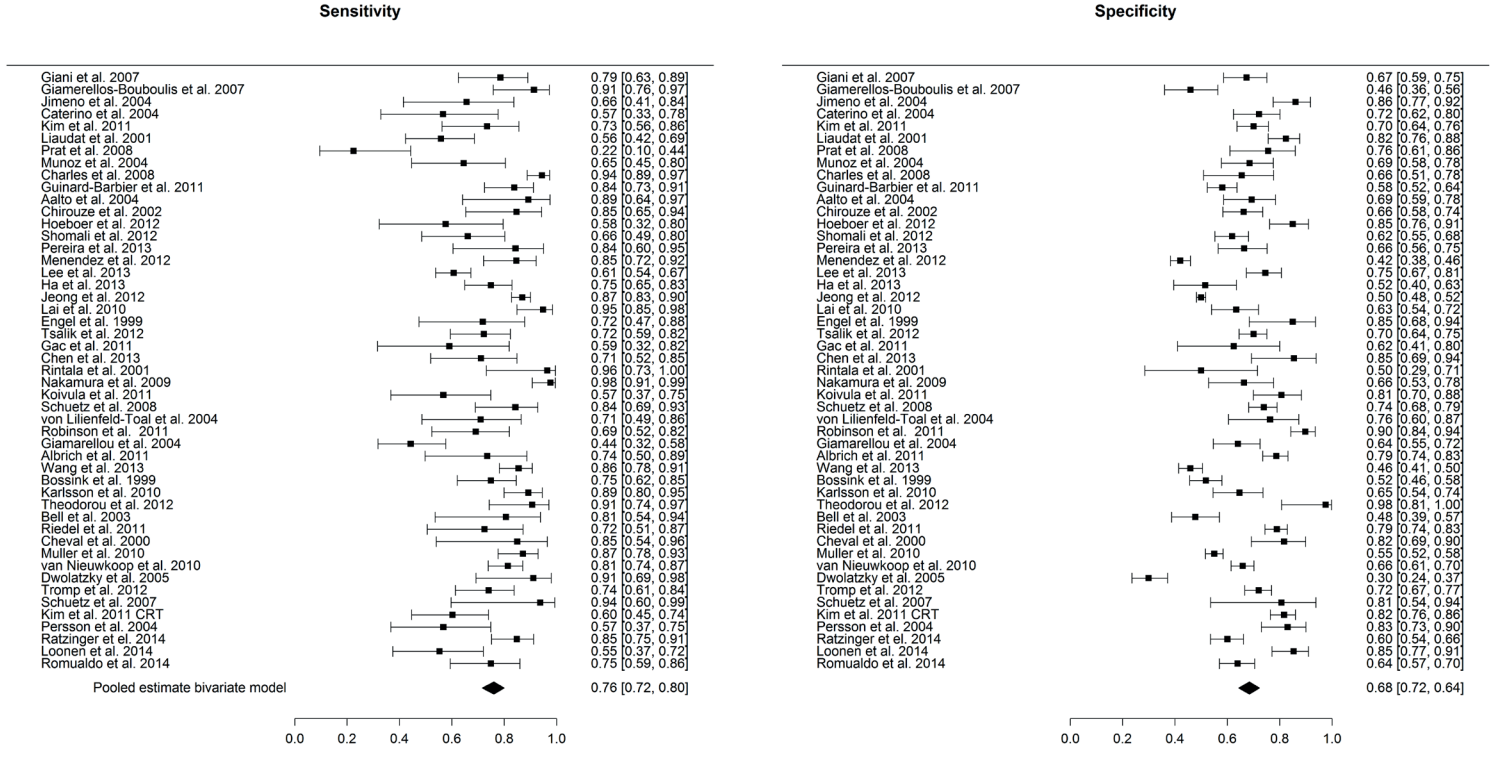


Figure 3. Accuracy estimates analysis for bacteraemia versus non-bacteraemia, including all studies (n=49).

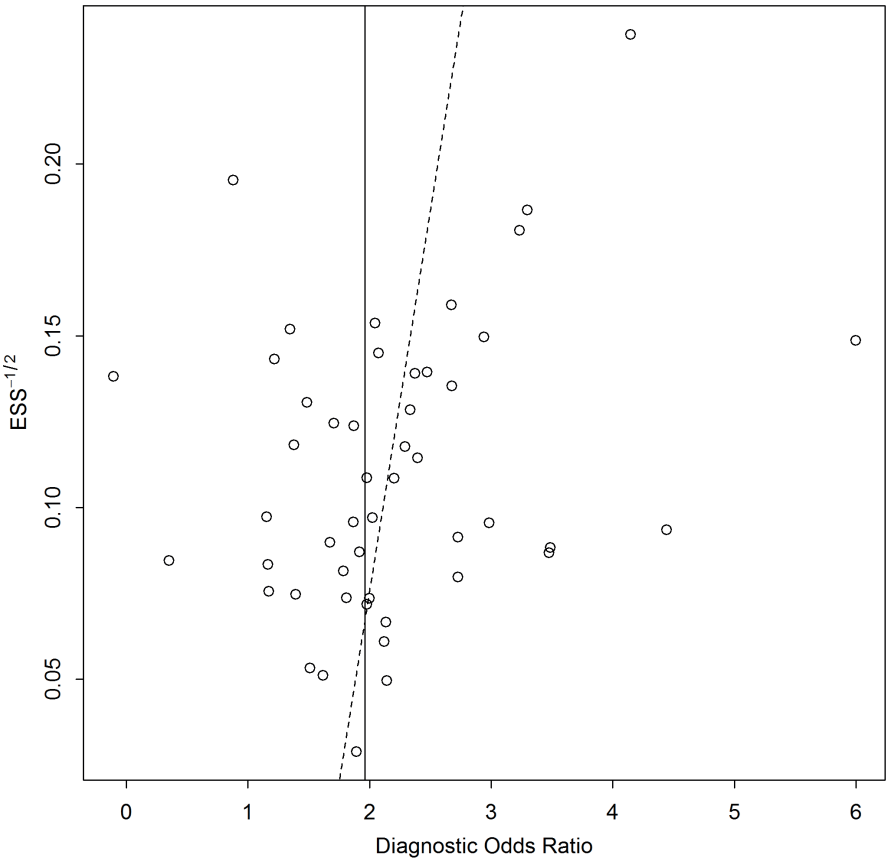


Figure 4. Evaluation of publication bias. The Deeks funnel plot assymetry test was non-significant (P=0.13). Individual studies are shown as open circles and the interrupted line represents the regression line. ESS = effective sample size.

SROC (0.88) in ICU patients. The lowest sensitivity was found in immunocompromised/neutropenic patients (66), the highest in ICU patients (89). The lowest specificity was found in patients with localised infections (55) and the highest in immunocompromised/neutropenic patients (78). Table 6 shows the 2x2 tables with low positive predictive values (17-28%) and high negative predictive values (95-98%) for different hospital settings at the 0.5 ng/mL procalcitonin cut-off. There was significant heterogeneity in the overall analysis and in most subgroups (Table 4). However, there was no indication of a threshold-effect.

Evaluation of publication bias

Figure 4 displays the Deeks funnel plot asymmetry test of this meta-analysis. The Deeks test was not statistically significant (P=0.13) indicating that there is no direct evidence for publication bias.

Discussion

This study evaluates the diagnostic accuracy of procalcitonin for bacteraemia in different subgroups of adult hospitalised patients suspected of infection or sepsis. Overall, at a cut-off level of 0.5 ng/mL, procalcitonin had a fair diagnostic accuracy for bacteraemia with an SROC of 0.79. The pooled AUC values of procalcitonin for the diagnosis bacteraemia in subgroups ranged from 0.71-0.88, with sensitivities ranging from 66% in immunocompromised/neutropenic patients to 89% in ICU patients and specificities ranging from 55% in bacteraemia vs. local infections to 78% in immunocompromised/neutropenic patients. Based on these results low procalcitonin levels in particular can be used to rule out the presence of bacteraemia.

Two previous meta-analyses on the diagnostic accuracy of procalcitonin for sepsis had contradicting conclusions while having comparable results [12,18]. Tang et al. concluded that there was no clear use for procalcitonin in diagnosing sepsis (area under the SROC of 0.78, sensitivity of 71% and specificity of 70%) [12]. However, their inclusion may be biased by specifically excluding sepsis originating from certain types of common infection sites [12]. In contrast, Wacker et al. concluded that procalcitonin was useful for the diagnosis of sepsis (area under the SROC 0.85, sensitivity 77%, specificity 79%) [18]. They included studies on adult and paediatric patients comparing sepsis to SIRS. Sepsis, however, was defined as clinically suspected or microbiologically proven infection [18]. Two other meta-analyses studying the diagnostic use of procalcitonin for bacterial infection found an area under the SROC curve ranging from 0.82-0.89, sensitivity 83-88%, specificity 81-83% [28,34]. Both analyses had comparable results but again contradicting conclusions. Simon et al. compared CRP and procalcitonin in a meta-analysis on the diagnostic accuracy in either proven or suspected bacterial infection, favouring PCT to be used in clinical practice [28]. In contrast, Lee et al. contented that PCT should not be used as single diagnostic

tool for infection [34]. However their conclusion was based on only four studies on the diagnostic accuracy of procalcitonin for bacterial infection in elderly patients [34]. As far as we know there is only one previous meta-analysis on the diagnostic accuracy of procalcitonin for bacteraemia with an area under the SROC of 0.84, sensitivity 76%, specificity 70% [40]. This study concluded that widespread use of procalcitonin is not recommended because of the moderate diagnostic accuracy of PCT to predict bacteraemia [40]. This conclusion was based on 17 included studies of which not all contained bacteraemia as primary endpoint. Even though previous meta-analyses showed similar results their conclusion differ, possibly due to differences in interpretation of clinically useful AUC values. In contrast to our study, the above-mentioned meta-analyses only used a small selection of the available literature or used sepsis syndrome and not microbiologically documented infection as their endpoint. Our study shows that procalcitonin can be used in the diagnostic process of bacteraemia regardless of its clinical symptoms. As shown in Table 6 low procalcitonin levels can be used to rule out the presence of bacteraemia in different clinical settings.

This meta-analysis has several limitations. There is some evidence for a concentration-response relation between procalcitonin levels and probability of infection and disease severity [52]. The definition of our primary outcome measure, bacteraemia, does not acknowledge such a concentration-response relation. Only a minority of the studies in this meta-analysis formally excluded patients treated with antibiotics prior to inclusion [23,24,53-57]. We cannot be certain that false negative results, due to possible antibiotic treatment prior to inclusion, led to underestimation of the effect. Even though the effect size is only fair (area under the SROC 0.79) its direction is positive in almost all studies, in spite of heterogeneity. High I-squares are to be expected because of the variation in cut-offs used in the different included studies and sensitivity and specificity both depend on cut-offs. To homogenise the results we attempted to use the sensitivities and specificities corresponding with the cut-off value closest to 0.5 ng/mL if multiple cut-off values were given. Other potential factors that could have contributed to heterogeneity are variety in inclusion criteria, underlying diseases, co-morbidities, clinical course and treatment prior to inclusion, variety in the control groups used for comparison against bacteraemia, department of sample collection, and differences in test performance of the various procalcitonin assays. In order to reduce the influence of these factors on heterogeneity we

performed analyses in the supposedly more homogeneous patient subgroups. As to be expected, substantial heterogeneity remained in most subgroups. A Funnel plot analysis based on the standard error of the lnDOR can be misleading, therefore we evaluated potential publication bias using the recommended effective sample size-based funnel plots and associated regression tests of asymmetry according to Deeks [51].

Conclusions

In conclusion, this systematic review and meta-analysis shows that procalcitonin has a fair diagnostic accuracy for bacteraemia in adult, hospitalised patients suspected of infection or sepsis. In particular low procalcitonin levels can be used to rule out the presence of bacteraemia. Further research on the safety and efficacy of using procalcitonin as a single diagnostic tool to withhold taking blood cultures remains to be proven.

References

1. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006;34:1589-96.
2. Wang HE, Szychowski JM, Griffin R, et al. Long-term mortality after community-acquired sepsis:a longitudinal population-based cohort study. BMJ Open 2014;4:e004283.
3. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock, 2012. Intensive Care Med 2013;39:165-228.
4. Kopterides P, Siempos II, Tsangaris I, et al. Procalcitonin-guided algorithms of antibiotic therapy in the intensive care unit: a systematic review and meta-analysis of randomized controlled trials. Crit Care Med 2010;38:2229-41.
5. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992;101:1644-55.
6. Harbarth S, Holeckova K, Froidevaux C, et al. Diagnostic value of procalcitonin, interleukin-6, and interleukin-8 in critically ill patients admitted with suspected sepsis. Am J Respir Crit Care Med 2001;164:396-402.
7. Pettila V, Hynninen M, Takkunen O, et al. Predictive value of procalcitonin and interleukin 6 in critically ill patients with suspected sepsis. Intensive Care Med 2002;28:1220-5.
8. Hensler T, Sauerland S, Lefering R, et al. The clinical value of procalcitonin and neopterin in predicting sepsis and organ failure after major trauma. Shock 2003;20:420-6.
9. Riedel S. Procalcitonin and the role of biomarkers in the diagnosis and management of sepsis. Diagnostic Microbiol and Inf Dis 2012;73:221-227.
10. Wang H, Yin F, Shen DX, et al. Predictive value of procalcitonin for excluding bloodstream infection: results of a retrospective study and utility of a rapid, quantitative test for procalcitonin. J Int Med Res 2013;41:1671-81.
11. Uzzan, B., et al., Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. Crit Care Med 2006;34:1996-2003.
12. Tang BM, Eslick GD, Craig JC, et al. Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. Lancet Infect Dis 2007;7:210-7.
13. Sakr Y, Sponholz C, Tuche F, Brunkhorst F, Reinhart K. The role of procalcitonin in febrile neutropenic patients: review of the literature. Infection 2008;36:396-407.
14. Yu Z, Liu J, Sun Q, Qiu Y, Han S, Guo X. The accuracy of the procalcitonin test for the diagnosis of neonatal sepsis: a meta-analysis. Scand J Infect Dis 2010;42:723-33.
15. Mann ea, Wood GL, Wade CE. Use of procalcitonin for the detection of sepsis in the critically ill burn patient: a systematic review of the literature. Burns 2011;37:549-58.
16. Vouloumanou EK, Plessa E, Karageorgopoulos DE, Mantadakis E, Falagas ME. Serum procalcitonin as a diagnostic marker for neonatal sepsis: a systematic review and meta-analysis. Intensive Care Med 2011;5:2174-2178.
17. Haeulser GM, Carlesse F, Phililips RS. An updated systematic review and meta-analysis of the predicitve value of serum biomarkers in the assessment of fever during neutropenia in children with cancer. Pediatr Infect Dis J 2013;10:e390-396.
18. Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect Dis 2013;13:426-35.
19. Bates DW, Sands K, Miller E, et al. Predicting bacteremia in patients with sepsis syndrome. Academic Medical Center Consortium Sepsis Project Working Group. J Infect Dis 1997;176:1538-51.

20. Sands KE, Bates DW, Lanken PN, et al. Epidemiology of sepsis syndrome in 8 academic medical centers. JAMA 1997;278:234-40.
21. Magadia RR. Weinstein MP. Laboratory diagnosis of bacteremia and fungemia. Infect Dis Clin North Am 2001;15:1009-24.
22. Flayhart D, Borek AP, Wakefield T, Dick J, Carroll KC. Comparison of BACTEC PLUS blood culture media to BacT/Alert FA blood culture media for detection of bacterial pathogens in samples containing therapeutic levels of antibiotics. J Clin Microbiol 2007;45:816-21.
23. Muller F, Christ-Crain M, Bregenzer T, et al. Procalcitonin levels predict bacteremia in patients with community-acquired pneumonia: a prospective cohort trial. Chest 2010;138:121-9.
24. Kim MH, Lim G, Kang SY, Lee WI, Suh JT, Lee HJ. Utility of procalcitonin as an early diagnostic marker of bacteremia in patients with acute fever. Yonsei Med J 2011;52:276-81.
25. Lai CC, Chen SY, Wang CY, et al. Diagnostic value of procalcitonin for bacterial infection in elderly patients in the emergency department. J Am Geriatr Soc 2010;58:518-22.
26. Schuetz P, Mueller B, Trampuz A. Serum procalcitonin for discrimination of blood contamination from bloodstream infection due to coagulase-negative staphylococci. Infection 2007;35:352-5.
27. Riedel S, Melendez JH, An AT, Rosenbaum JE, Zenilman JM. Procalcitonin as a marker for the detection of bacteremia and sepsis in the emergency department. Am J Clin Pathol 2011;135:182-9.
28. Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis 2004;39:206-17.
29. Van den Breul, Thompson MJ, Haj-Hassan T, et al. Diagnostic value of laboratory tests in identifying serious infections in febrile children: systematic review. BMJ 2011;342:d3082.
30. Lin SG, Hou TY, Huang DH, et al. Role of procalcitonin in the diagnosis of severe infection in pediatric patients with fever and Neutropenia--a systemic review and meta-analysis. Pediatr Infect Dis J 2012;31: e182-8.
31. Wu JY, Lee SH, Shen CJ, et al. Use of serum procalcitonin to detect bacterial infection in patients with autoimmune diseases: a systematic review and meta-analysis. Arthritis Rheum 2012;64:3034-42.
32. Yo CH, Hsieh PS, Lee SH, et al. Comparison of the test characteristics of procalcitonin to C-reactive protein and leukocytosis for the detection of serious bacterial infections in children presenting with fever without source: a systematic review and meta-analysis. Ann Emerg Med 2012;60:591-600.
33. Dou YH, Du JK, Liu HL, Shong XD. The role of procalcitonin in the identification of invasive fungal infection-a systemic review and meta-analysis. Diagn Microbiol Infect Dis 2013;76:464-9.
34. Lee SH, Chan RC, Wu JY, Chen HW, Chang SS, Lee CC. Diagnostic value of procalcitonin for bacterial infection in elderly patients – a systemic review and meta-analysis. Int J Clin Pract 2013;67:1350-1357.
35. Shen CJ, Wu MS, Lin KH, et al. The use of procalcitonin in the diagnosis of bone and joint infection: a systemic review and meta-analysis. Eur J Clin Microbiol Infect Dis 2013;32:807-814.
36. Yu CW, Juan LI, Hsu SC, Chen CK, Wu CW, Lee CC, Wu JY. Role of procalcitonin in the diagnosis of infective endocarditis: a meta-analysis. Am J Emerg Med 2013;31:935-41.
37. Huang SL, Lee HC, Yu CM, et al. Value of procalcitonin in differentiating pulmonary tuberculosis from other pulmonary infections: a meta-analysis. Int J Tuberc Lung Dis 2014;18:470-477.
38. Pfister R, Kochanek M, Leygeber T, et al. Procalcitonin for diagnosis of bacterial pneumonia in critically ill patients during 2009 H1N1 influenza pandemic: a prospective cohort study, systematic review and individual patient data meta-analysis. Crit Care 2014;10:R44.
39. Lin KH, Wang FL, Wu MS, et al. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection in patients with liver cirrhosis: a systematic review and meta-analysis. Diagn Microbiol Infect Dis 2014;21:S0732-8893.

40. Jones AE, Fiechtl JF, Brown MD, Ballew JJ, Kline JA. Procalcitonin test in the diagnosis of bacteremia: a meta-analysis. Ann Emerg Med 2007;50:34-41.
41. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009;339:b2700.
42. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol 2003;3:25.
43. Leeflang MM, Deeks JJ, Gatsonis C, Bossuyt. Systematic reviews of diagnostic test accuracy. Ann Intern Med, 2008. 149:889-97.
44. Harbord RM, Whiting P, metandi: Meta-analysis of diagnostic accuracy using hierarchical logistic regression. Stata Journal 2009;9:211-229.
45. Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol 2005;58:982-90.
46. Lai CC, Tan CK, Chen SY, et al. Diagnostic value of procalcitonin for bacteremia in patients with bacterial infection at the emergency department. J Infection 2010;61:512-5.
47. Mueller F, Christ-Crain M, Bregenzer T, et al. Procalcitonin levels predict bacteremia in patients with community-acquired pneumonia: a prospective cohort trial. Chest 2010;138:121-9.
48. Fazili T, Endy T, Javaid W, et al. Role of procalcitonin in guiding antibiotic therapy. Am J Health Syst Pharm 2012;69:2057-2061.
49. Quenot JP, Luyt CE, Roche N, et al. Role of biomarkers in the management of antibiotic therapy: an expert panel review II: clinical use of biomarkers for initiation or discontinuation of antibiotic therapy. Ann Int Care 2013;3:21.
50. Tape TG. Interpreting diagnostic tests. http://gim.unmc.edu/dxtests/Default.htm. Accessed October 12, 2014.
51. Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol 2005;58:882-893.
52. Schuetz P, AlbrichW, Mueller B. Procalcitonin for diagnosis of infection and guide to antibiotic decisions: past, present and future. BMC Med 2011;9:107.
53. Guinard-Barbier S, Grabar S, Chenevier-Gobeaux C, et al. Is mid-regional pro-atrial natriuretic peptide (MRproANP) an accurate marker of bacteremia in pyelonephritis? Biomarkers 2011;16:355-63.
54. Hoenigl M, Raggam RB, Wagner J, et al. Diagnostic accuracy of soluble urokinase plasminogen activator receptor (suPAR) for prediction of bacteremia in patients with systemic inflammatory response syndrome. Clin Biochem 2013;46:225-9.
55. Menendez R, Sahuquillo-Arce JM, Reyes S, et al. Cytokine activation patterns and biomarkers are influenced by microorganisms in community-acquired pneumonia. Chest 2012;141:1537-45.
56. Park JH, Wee JH, Choi SP, Oh SH. The value of procalcitonin level in community-acquired pneumonia in the ED. Am J Emerg Med 2012;30:1248-54.
57. Theodorou VP, Papaioannou VE, Tripsianis GA, et al. Procalcitonin and procalcitonin kinetics for diagnosis and prognosis of intravascular catheter-related bloodstream infections in selected critically ill patients: a prospective observational study. BMC Infect Dis 2012;12:247.
58. Aalto H, Takala A, Kautiainen H, Repo H. Laboratory markers of systemic inflammation as predictors of bloodstream infection in acutely ill patients admitted to hospital in medical emergency. Eur J Clin Microbiol Infect Dis 2004;23:699-704.

59. Albrich WC, Mueller B. Predicting bacteremia by procalcitonin levels in patients evaluated for sepsis in the emergency department. Expert Rev Anti Infect Ther 2011;9:653-6.
60. Bell K, Wattie M, Byth K, et al. Procalcitonin: a marker of bacteraemia in SIRS. Anaesth Int Care 2003;31:629-36.
61. Bogar L, Molnar Z, Kenyeres P, Tarsoly P. Sedimentation characteristics of leucocytes can predict bacteraemia in critical care patients. J Clin Pathol 2006;59:523-5.
62. Bossink AW, Groeneveld AB, Thijs TG. Prediction of microbial infection and mortality in medical patients with fever: plasma procalcitonin, neutrophilic elastase-alpha1-antitrypsin, and lactoferrin compared with clinical variables. Clin Infect Dis 1999;29:398-407.
63. Caterino JM, Scheatzle MD, Forbes ML, D'Antonio JA. Bacteremic elder emergency department patients: procalcitonin and white count. Acad Emerg Med 2004;11:393-6.
64. Charles PE, Ladoire S, Snauwaert A, et al. Impact of previous sepsis on the accuracy of procalcitonin for the early diagnosis of blood stream infection in critically ill patients. BMC Infect Dis 2008;8:163.
65. Chen J, Wang Y, Shen Z, Zhu Z, Song Y, Han R. Early diagnostic value of plasma PCT and BG assay for CRBSI after OLT. Transplant Proc 2011;43:1777-9.
66. Cheval C, Timsit JF, Garrouste-Orgeas M, et al. Procalcitonin (PCT) is useful in predicting the bacterial origin of an acute circulatory failure in critically ill patients. Intensive Care Med 2000;26 (Suppl 2):S153-8.
67. Chirouze C, Schuhmacher H, Rabaud C, et al. Low serum procalcitonin level accurately predicts the absence of bacteremia in adult patients with acute fever. Clin Infect Dis 2002;35:156-61.
68. Dwolatzky T, Olshtain-Pops K, Yinnon AM, et al. Procalcitonin in the elderly: normal plasma concentrations and response to bacterial infections. Eur J Clin Microbiol Infect Dis 2005;24:763-5.
69. Engel A, Steinbach G, Kern P, Kern WV. Diagnostic value of procalcitonin serum levels in neutropenic patients with fever: comparison with interleukin-8. Scand J Infect Dis 1999;31:185-9.
70. Gac AC, Parienti JJ, Chantepie S, Fradin S, Le Coutour X, Leclercq R, Reman O. Dynamics of procalcitonin and bacteremia in neutropenic adults with acute myeloid leukemia. Leuk Res 2011;35:1294-6.
71. Gaini S, Koldkjaer OG, Møller HJ, Pedersen C, Pedersen SS. A comparison of high-mobility group-box 1 protein, lipopolysaccharide-binding protein and procalcitonin in severe community-acquired infections and bacteraemia: a prospective study. Crit Care 2007;11:R76.
72. Giamarellos-Bourboulis EJ, Grecka P, Poulakou G, Anargyrou K, Katsilambros N, Giamarellou H. Assessment of procalcitonin as a diagnostic marker of underlying infection in patients with febrile neutropenia. Clin Infect Dis 2001;32:1718-25.
73. Giamarellou H, Giamarellos-Bourboulis EJ, Repoussis P, et al. Potential use of procalcitonin as a diagnostic criterion in febrile neutropenia: experience from a multicentre study. Clin Microbiol Infect 2004;10:628-33.
74. Ha YE, Kang CI, Wi YM, et al. Diagnostic usefulness of procalcitonin as a marker of bacteremia in patients with acute pyelonephritis. Scand J Clin Lab Invest 2013;73:444-8.
75. Hoeboer SH, Alberts E, van den Hul I, Tacx AN, Debets-Ossenkopp YJ, Groeneveld AB. Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: a case for procalcitonin. J Infect 2012;64:484-93.
76. Hoenigl M, Raggam RB, Wagner J, et al. Procalcitonin fails to predict bacteremia in SIRS patients: a cohort study. Int J Clin Pract 2014;doi 10.1111:12474.
77. Jeong S, Park Y, Cho Y, Kim HS. Diagnostic utilities of procalcitonin and C-reactive protein for the prediction of bacteremia determined by blood culture. Clin Chim Acta 2012;413:1731-6.
78. Jimeno A, García-Velasco A, del Val O, et al. Assessment of procalcitonin as a diagnostic and prognostic marker in patients with solid tumors and febrile neutropenia. Cancer 2004;100:2462-9.

79. Kallio R, Surcel HM, Bloigu A, Syrjälä H. C-reactive protein, procalcitonin and interleukin-8 in the primary diagnosis of infections in cancer patients. Eur J Cancer 2000;36:889-94.
80. Karlsson S, Heikkinen M, Pettilä V, et al. Predictive value of procalcitonin decrease in patients with severe sepsis: a prospective observational study. Crit Care 2010;14:R205.
81. Kim DY, Lee YS, Ahn S, Chun YH, Lim KS. The usefulness of procalcitonin and C-reactive protein as early diagnostic markers of bacteremia in cancer patients with febrile neutropenia. Cancer Res Treat 2011;43:176-80.
82. Koivula I, Hämäläinen S, Jantunen E, et al. Elevated procalcitonin predicts Gram-negative sepsis in haematological patients with febrile neutropenia. Scand J Infect Dis 2011;43:471-8.
83. Lee CH, Kim J, Park Y, et al. Delta neutrophil index discriminates true bacteremia from blood culture contamination. Clin Chim Acta 2014;427:11-4.
84. Liaudat S, Dayer E, Praz G, Bille J, Troillet N. Usefulness of procalcitonin serum level for the diagnosis of bacteremia. Eur J Clin Microbiol Infect Dis 2001;20:524-7.
85. Loonen AJM, De Jager CPC, Tosserams J, et al. Biomarkers and molecular analysis to improve bloodstream infection diagnostics in an emergency care unit. PLoS One 2014;9:e87315.
86. Mencacci A, Leli C, Cardaccia A, et al. Procalcitonin predicts real-time PCR results in blood samples from patients with suspected sepsis. PLoS One 2012;7:e53279.
87. Munoz P, Simarro N, Rivera M, Alonso R, Alcalá L, Bouza E. Evaluation of procalcitonin as a marker of infection in a nonselected sample of febrile hospitalized patients. Diagn Microbiol Infect Dis 2004;49:237-41.
88. Nakamura A, Wada H, Ikejiri M, et al, Efficacy of procalcitonin in the early diagnosis of bacterial infections in a critical care unit. Shock 2009;31:586-91.
89. van Nieuwkoop C, Bonten TN, van't Wout JW, et al. Procalcitonin reflects bacteremia and bacterial load in urosepsis syndrome: a prospective observational study. Crit Care 2010;14:R206.
90. Pereira JM, Teixeira-Pinto A, Basílio C, Sousa-Dias C, Mergulhão P, Paiva JA. Can we predict pneumococcal bacteremia in patients with severe community-acquired pneumonia? J Crit Care 2013;28:970-4.
91. Persson L, Engervall P, Magnuson A, et al. Use of inflammatory markers for early detection of bacteraemia in patients with febrile neutropenia. Scand J Infect Dis 2004;36:365-71.
92. Prat C, Sancho JM, Dominguez J, et al. Evaluation of procalcitonin, neopterin, C-reactive protein, IL-6 and IL-8 as a diagnostic marker of infection in patients with febrile neutropenia. Leuk Lymphoma 2008;49:1752-61.
93. Ratzinger F, Schuardt M, Eichbichler K, et al. Utility of sepsis biomarkers and the infection probability score to discriminate sepsis and systemic inflammatory response syndrome in standard care patients. PLoS One 2013;8:e82946.
94. Rintala EM, Aittoniemi J, Laine S, Nevalainen TJ, Nikoskelainen J. Early identification of bacteremia by biochemical markers of systemic inflammation. Scand J Clin Lab Invest 2001;61:523-30.
95. Robinson JO, Lamoth F, Bally F, Knaup M, Calandra T, Marchetti O. Monitoring procalcitonin in febrile neutropenia: what is its utility for initial diagnosis of infection and reassessment in persistent fever? PLoS One 2011;6:e18886.
96. Romualdo LG, Torrella PE, Gonzalez MV, et al. Diagnostic accuracy of prepepsin (soluble CD 14 subtype) for prediction of bacteremia in patients with systemic inflammatory response syndrome in the emergency department. Clin Biochem 2014;47:505-508.
97. Schuetz P, Stolz D, Mueller B, et al. Endothelin-1 precursor peptides correlate with severity of disease and outcome in patients with community acquired pneumonia. BMC Infect Dis, 2008. 8:22.
98. Shi Y, DU B, Xu YC, Rui X, DU W, Wang Y. Early changes of procalcitonin predict bacteremia in patients with intensive care unit-acquired new fever. Chin Med J 2013;126:1832-7.

99. Shomali W, Hachem R, Chaftari AM, et al. Can procalcitonin distinguish infectious fever from tumor-related fever in non-neutropenic cancer patients? Cancer 2012;118:5823-9.
100. Su CP, Chen TH, Chen SY, et al. Predictive model for bacteremia in adult patients with blood cultures performed at the emergency department: a preliminary report. J Microbiol Immunol Infect 2011;44:449-55.
101. Suarez-Santamaria M, et al. Prognostic value of inflammatory markers (notably cytokines and procalcitonin), nutritional assessment, and organ function in patients with sepsis. Eur Cytokine Netw 2010;21:19-26.
102. Tromp M, Lansdorp B, Bleeker-Rovers CP, Gunnewiek JM, Kullberg BJ, Pickkers P. Serial and panel analyses of biomarkers do not improve the prediction of bacteremia compared to one procalcitonin measurement. J Infect 2012;65:292-301.
103. Tsalik EL, Jaggers LB, Glickman SW, et al. Discriminative value of inflammatory biomarkers for suspected sepsis. J Emerg Med 2012;43:97-106.
104. Vanska M, Koivula I, Jantunen E, et al. IL-10 combined with procalcitonin improves early prediction of complications of febrile neutropenia in hematological patients. Cytokine 2012;60:787-92.
105. von Lilienfeld-Toal M, Dietrich MP, Glasmacher A, et al. Markers of bacteremia in febrile neutropenic patients with hematological malignancies: procalcitonin and IL-6 are more reliable than C-reactive protein. Eur J Clin Microbiol Infect Dis 2004;23:539-44.
106. Adamzik M, Eggmann M, Frey UH, et al. Comparison of thromboelastometry with procalcitonin, interleukin 6, and C-reactive protein as diagnostic tests for severe sepsis in critically ill adults. Crit Care 2010;14:R178.
107. Barati M, Alinejad F, Bahar MA, et al. Comparison of WBC, ESR, CRP and PCT serum levels in septic and non-septic burn cases. Burns 2008;34:770-4.
108. Bele N, Darmon M, Coquet I, et al. Diagnostic accuracy of procalcitonin in critically ill immunocompromised patients. BMC Infect Dis 2011;11:224
109. Bugden SA, Coles C, Mills GD. The potential role of procalcitonin in the emergency department management of febrile young adults during a sustained meningococcal epidemic. Emerg Med Australas 2004;16:114-9.
110. Delevaux I, André M, Colombier M, et al. Can procalcitonin measurement help in differentiating between bacterial infection and other kinds of inflammatory processes? Ann Rheum Dis 2003;62:337-40.
111. Fluri F, Morgenthaler NG, Mueller B, Christ-Crain M, Katan M. Copeptin, procalcitonin and routine inflammatory markers-predictors of infection after stroke. PLoS One 2012;7:e48309.
112. Freund, Y, Delerme S, Goulet H, Bernard M, Riou B, Hausfater P. Serum lactate and procalcitonin measurements in emergency room for the diagnosis and risk-stratification of patients with suspected infection. Biomarkers 2012;17:590-6.
113. Hettwer S, Wilhelm J, Schürmann M, et al. Microbial diagnostics in patients with presumed severe infection in the emergency department. Med Klin Intensivmed Notfmed 2012;107:53-62.
114. Jereb M, Kotar T, Jurca T, Lejko Zupanc T. Usefulness of procalcitonin for diagnosis of infective endocarditis. Intern Emerg Med 2009;4:221-6.
115. van Langevelde P, Joop K, van Loon J, et al. Endotoxin, cytokines, and procalcitonin in febrile patients admitted to the hospital: identification of subjects at high risk of mortality. Clin Infect Dis 2000;31:1343-8.
116. Magrini L, Travaglino F, Marino R, et al. Procalcitonin variations after Emergency Department admission are highly predictive of hospital mortality in patients with acute infectious diseases. Eur Rev Med Pharmacol Sci 2013;17:133-42.

117. Oberhoffer M, Russwurm S, Bredle D, Chatzinicolaou K, Reinhart K. Discriminative power of inflammatory markers for prediction of tumor necrosis factor-alpha and interleukin-6 in ICU patients with systemic inflammatory response syndrome (SIRS) or sepsis at arbitrary time points. Intensive Care Med 2000;26:S170-4.
118. Patil VK, Morjaria JB, De Villers F, Babu SK. Associations between procalcitonin and markers of bacterial sepsis. Medicina (Kaunas) 2012;48:383-7.
119. Pizzolato E, Ulla M, Galluzzo C, et al. Role of presepsin for the evaluation of sepsis in the emergency department. Clin Chem Lab Med 2014;10.1515;2014-0199.
120. Quiroga B, Villaverde M, Vega A, Abad S, Reque J, López-Gómez JM. Procalcitonin as an early predictor of acute infection in hemodialysis patients. Nefrologia 2014;34:341-6.
121. Reynolds SC, Shorr AF, Muscedere J, Jiang X, Heyland DK. Longitudinal changes in procalcitonin in a heterogeneous group of critically ill patients. Crit Care Med 2012;40:2781-7.
122. Rowther FB, Rodrigues CS, Deshmukh MS, et al. Prospective comparison of eubacterial PCR and measurement of procalcitonin levels with blood culture for diagnosing septicemia in intensive care unit patients. J Clin Microbiol 2009;47:2964-9.
123. Sakr Y, Burgett U, Nacul FE, Reinhart K, Brunkhorst F. Lipopolysaccharide binding protein in a surgical intensive care unit: a marker of sepsis? Crit Care Med 2008;36:2014-22.
124. Stankovic Stojanovic K, Steichen O, Lionnet F, et al. Is procalcitonin a marker of invasive bacterial infection in acute sickle-cell vaso-occlusive crisis? Infection 2011;39:41-5.
125. Steichen O, Bouvard E, Grateau G, Bailleul S, Capeau J, Lefèvre G. Diagnostic value of procalcitonin in acutely hospitalized elderly patients. Eur J Clin Microbiol Infect Dis 2009;28:1471-6.
126. Uusitalo-Seppala R, Koskinen P, Leino A, Peuravuori H, Vahlberg T, Rintala EM. Early detection of severe sepsis in the emergency room: diagnostic value of plasma C-reactive protein, procalcitonin, and interleukin-6. Scand J Infect Dis 2011;43:883-90.
127. Viallon A, Guyomarc'h S, Marjollet O, et al. Can emergency physicians identify a high mortality subgroup of patients with sepsis: role of procalcitonin. Eur J Emerg Med 2008;15:26-33.
128. Wunderink RG, Diederich ER, Caramez MP, et al. Rapid response team-triggered procalcitonin measurement predicts infectious intensive care unit transfers. Crit Care Med 2012;40:2090-5.
129. Yan L, Liao P, Xu LL, Zhao Y. Usefulness of procalcitonin in elderly patients with bacterial infection. Clin Lab 2014;60:139-42.
130. Zhu Y, Cao X, Zhang K, Xie W, Xu D, Zhong C. Delta Mean Neutrophil Volume (DeltaMNV) is Comparable to Procalcitonin for Predicting Postsurgical Bacterial Infection. J Clin Lab Anal 2014;doi 10.1001:21684.
131. Charles PE, Dalle F, Aho S, et al. Serum procalcitonin measurement contribution to the early diagnosis of candidemia in critically ill patients. Intensive Care Med 2006;32:1577-83.
132. Charles PE, Castro C, Ruiz-Santana S, León C, Saavedra P, Martín E. Serum procalcitonin levels in critically ill patients colonized with Candida spp: new clues for the early recognition of invasive candidiasis? Intensive Care Med 2009;35:2146-50.
133. Martini A, Gottin L, Menestrina N, Schweiger V, Simion D, Vincent JL. Procalcitonin levels in surgical patients at risk of candidemia. J Infect 2010;60:425-30.
134. Charles PE, Ladoire S, Aho S, et al. Serum procalcitonin elevation in critically ill patients at the onset of bacteremia caused by either Gram negative or Gram positive bacteria. BMC Infect Dis 2008;8:38.
135Knudsen JB, Fuursted K, Petersen E, et al. Procalcitonin in 759 patients clinically suspected of infective endocarditis. Am J Med 2010;123:1121-7.
136. Mueller C, Huber P, Laifer G, Mueller B, Perruchoud AP. Procalcitonin and the early diagnosis of infective endocarditis. Circulation 2004;109:1707-10.

137. Shomali W, Hachem R, Chaftari AM, et al. Can procalcitonin differentiate Staphylococcus aureus from coagulase-negative staphylococci in clustered gram-positive bacteremia? Diagn Microbiol Infect Dis 2013;76:158-61.
138. Ahn S, Lee YS, Chun YH, Lim KS, Kim W, Lee JL. Predictive factors of bacteraemia in low-risk patients with febrile neutropenia. Emerg Med J 2012;29:715-9.
139. Lee H, Lee YS, Jeong R, Kim YJ, Ahn S. Predictive factors of bacteremia in patients with febrile urinary tract infection: an experience at a tertiary care center. Infection 2014;42:669-674.
140. Al Shuaibi M, Bahu RR, Chaftari AM, et al. Pro-adrenomedullin as a novel biomarker for predicting infections and response to antimicrobials in febrile patients with hematologic malignancies. Clin Infect Dis 2013;56:943-50.
141. Aouifi A, Piriou V, Bastien O, et al. Usefulness of procalcitonin for diagnosis of infection in cardiac surgical patients. Crit Care Med 2000;28:3171-6
142. Bloos F, Sachse S, Kortgen A, et al. Evaluation of a polymerase chain reaction assay for pathogen detection in septic patients under routine condition: an observational study. PLoS One 2012;7:e46003.
143. Boussekey N, Leroy O, Georges H, Devos P, d'Escrivan T, Guery B. Diagnostic and prognostic values of admission procalcitonin levels in community-acquired pneumonia in an intensive care unit. Infection 2005;33:257-63.
144. Cuculi F, Toggweiler S, Auer M, der Maur ChA, Zuber M, Erne P. Serum procalcitonin has the potential to identify Staphylococcus aureus endocarditis. Eur J Clin Microbiol Infect Dis 2008;27:1145-9.
145. Endo S, Suzuki Y, Takahashi G, et al. Usefulness of presepsin in the diagnosis of sepsis in a multicenter prospective study. J Infect Chemother 2012;18:891-7.
146. Feld R.. Bloodstream infections in cancer patients with febrile neutropenia. Int J Antimicrob Agents 2008;32:S30-3.
147. Foushee JA, Hope NH, Grace EE. Applying biomarkers to clinical practice: a guide for utilizing procalcitonin assays. J Antimicrob Chemother 2012;67:2560-9.
148. Gille-Johnson P, Hansson KE, Gardlund B. Clinical and laboratory variables identifying bacterial infection and bacteraemia in the emergency department. Scand J Infect Dis 2012;44:745-52.
149. Groeneveld AB, Hack CE. The role of the innate immune response in hospital- versus community-acquired infection in febrile medical patients. Int J Infect Dis 2008;12:660-70.
150. Guven H, Altintop L, Baydin A, et al. Diagnostic value of procalcitonin levels as an early indicator of sepsis. Am J Emerg Med 2002;20:202-6.
151. Juutilainen A, Hämäläinen S, Pulkki K, et al. Biomarkers for bacteremia and severe sepsis in hematological patients with neutropenic fever: multivariate logistic regression analysis and factor analysis. Leuk Lymphoma 2011;52:2349-55.
152. Kim KE, Han JY. Evaluation of the clinical performance of an automated procalcitonin assay for the quantitative detection of bloodstream infection. Korean J Lab Med 2010;30:153-9.
153. de Kruif MD, Lemaire LC, Giebelen IA, et al. The influence of corticosteroids on the release of novel biomarkers in human endotoxemia. Intensive Care Med 2008;34:518-22.
154. Lehmann LE, Hunfeld KP, Steinbrucker M, et al. Improved detection of blood stream pathogens by real-time PCR in severe sepsis. Intensive Care Med 2010;36:49-56.
155. Lodes U, Bohmeier B, Lippert H, König B, Meyer F. PCR-based rapid sepsis diagnosis effectively guides clinical treatment in patients with new onset of SIRS. Langenbecks Arch Surg 2012;397:447-55.
156. Mauro MV, Cavalcanti P, Perugini D, Noto A, Sperlì D, Giraldi C. Diagnostic utility of LightCycler SeptiFast and procalcitonin assays in the diagnosis of bloodstream infection in immunocompromised patients. Diagn Microbiol Infect Dis 2012;73:308-11.
157. Peters RP, Twisk JW, van Agtmael MA, Groeneveld AB. The role of procalcitonin in a decision tree for prediction of bloodstream infection in febrile patients. Clin Microbiol Infect 2006;12:1207-13.

158. Previsdomini M Gini M, Cerutti B, Dolina M, Perren A. Predictors of positive blood cultures in critically ill patients: a retrospective evaluation. Croat Med J 2012;53:30-9.
159. Sandri MT, Passerini R, Leon ME, et al. Procalcitonin as a useful marker of infection in hemato-oncological patients with fever. Anticancer Res 2008;28:3061-5.
160. Scott LK, Grier LR, Arnold TC, Conrad SA. Serum procalcitonin concentration as a negative predictor of serious bacterial infection in acute sickle cell pain crisis. Med Sci Monit 2003;9:CR426-31.
161. Su L, Han B, Liu C, et al. Value of soluble TREM-1, procalcitonin, and C-reactive protein serum levels as biomarkers for detecting bacteremia among sepsis patients with new fever in intensive care units: a prospective cohort study. BMC Infect Dis 2012;12:157.
162. Svaldi M, Hirber J, Lanthaler AI, et al. Procalcitonin-reduced sensitivity and specificity in heavily leucopenic and immunosuppressed patients. Br J Haematol 2001;115:53-7.
163. Ugarte H, Silva E, Mercan D, De Mendonça A, Vincent JL. Procalcitonin used as a marker of infection in the intensive care unit. Crit Care Med 1999;27:498-504.
164. von Lilienfeld-Toal M, Lehmann LE, Raadts AD, et al. Utility of a commercially available multiplex real-time PCR assay to detect bacterial and fungal pathogens in febrile neutropenia. J Clin Microbiol 2009;47:2405-10.
165. Yilmaz G, Köksal I, Karahan SC, Mentese A. The diagnostic and prognostic significance of soluble urokinase plasminogen activator receptor in systemic inflammatory response syndrome. Clin Biochem 2011;44:1227-30.
166. Lavrentieva A, Papadopoulou S, Kioumis J, Kaimakamis E, Bitzani M. PCT as a diagnostic and prognostic tool in burn patients. Whether time course has a role in monitoring sepsis treatment. Burns 2012;38:356-63.
167. Gaini S, Pedersen SS, Koldkaer OG, Pedersen C, Moestrup SK, Møller HJ. New immunological serum markers in bacteraemia: anti-inflammatory soluble CD163, but not proinflammatory high mobility group-box 1 protein, is related to prognosis. Clin Exp Immunol 2008;151:423-31.
168. Kocazeybek B, Kucukoglu S, Oner YA. Procalcitonin and C-reactive protein in infective endocarditis: correlation with etiology and prognosis. Chemotherapy 2003;49:76-84.

Chapter 5

Procalcitonin to guide taking blood cultures in the intensive care unit

Patrick J. van der Geest[1], Mostafa Mohseni[1], Daan Nieboer[2] Servet Duran[3], and A.B. Johan Groeneveld[1]

[1] Department of Intensive Care Medicine, Erasmus Medical Center, Rotterdam, The Netherlands.
[2] Department of Public Health, Erasmus Medical Center, Rotterdam, The Netherlands.
[3] Department of Intensive Care Medicine, Maasstad ziekenhuis, Rotterdam, The Netherlands.

Clinical microbiology and infection

Abstract

Objectives
Bacteraemia increases morbidity and mortality of critically ill patients; a fast and accurate diagnosis is therefore crucial. A low plasma procalcitonin (PCT) may virtually and rapidly rule out bacteraemia and its use could lead to a cost reduction by saving blood cultures. We therefore aimed to study the safety and efficacy of PCT in guiding blood culture taking in critically ill patients with suspected infection.

Design
Multi-center, cluster-randomized, cross-over trial in critically ill patients between January 2013 and September 2014. Patients suspected of infection by the attending intensivist in whom taking blood for culture was indicated were included. Patients in the participating intensive care units, stratified and randomized by regimen, were divided in a control group (standard of care) and a PCT-guided group.

Intervention
In both groups blood was drawn at the same moment for a PCT measurement and blood cultures. In the PCT-guided group, the attending intensivist sent blood cultures to the department of medical microbiology based on the result of the PCT measurement. Blood cultures were not sent if the PCT was below 0.25 ng/ml, unless otherwise indicated. In the control group, PCT results were available prior to analysis only.

Setting
Critically ill patients in the intensive care unit.

Subjects
The study included 564 patients who were suspected for having infection.

Measurements and main results
Two hundred eighty-eight patients were included in the control group and 276 patients in the PCT-guided group. No differences were seen in baseline

characteristics. The occurrence of bacteraemia was similar for both groups. The negative predictive value of PCT <0.25 ng/mL for bacteraemia was 96%. In the PCT-guided group 18 sets of blood cultures were saved in 17 patients. The intention to treat analysis showed a hazard ratio of 0.85 (95% CI, 0.62-1.17) and 0.89 (95% CI, 0.67-1.17) for 28- day and 90-day mortality respectively. The results were deemed non-inferior since the upper limit of the 95% CI was below the margin of 1.20. Using the PCT strategy can save €1.14 per suspected infection episode.

Conclusion

Applying PCT to guide blood cultures in critically ill patients with suspected infection seems to be safe and (cost-)effective.

Introduction

Critical illness predisposes to bacteraemia thereby increasing morbidity and mortality [1,2], particularly when diagnosis and administration of antibiotics are delayed [3,4]. Indeed, culturing costs time, and only 15-25% of blood cultures taken in critically ill patients suspected of infection prove positive, suggestive of a waste of resources [5]. The use of biomarkers, including procalcitonin (PCT), has been studied to improve a fast and accurate diagnosis of sepsis and bacteraemia with varying results [4,6-8]. However, we recently performed a meta-analysis of studies suggesting that a normal PCT has 96% negative predictive value for bacteremia [9]. Based on nine studies, the area under the receiver operating characteristic curve of PCT for bacteraemia in critically ill patients was 0.88 [6,9-17]. The studies included, however, were relatively small [10-12, 15-17] and not primarily designed to rule out or detect bacteraemia [6,10,12-14]. Nevertheless, a rapidly available and normal PCT might allow early prediction of negative blood cultures when blood sampling is clinically indicated for suspicion of infection and thereby avoid unnecessary blood culturing.

In the hypothesis that a normal PCT can be used to predict absence of bacteraemia in critically ill patients, we aimed to study the usefulness of a rapidly determined PCT, in saving blood cultures in critically ill patients in whom taking blood for culture is clinically indicated because of a suspicion of infection. We hypothesized that such a strategy can be safely applied in critically ill patients without increasing morbidity and mortality and is (cost-) effective.

Methods

Patients and study design

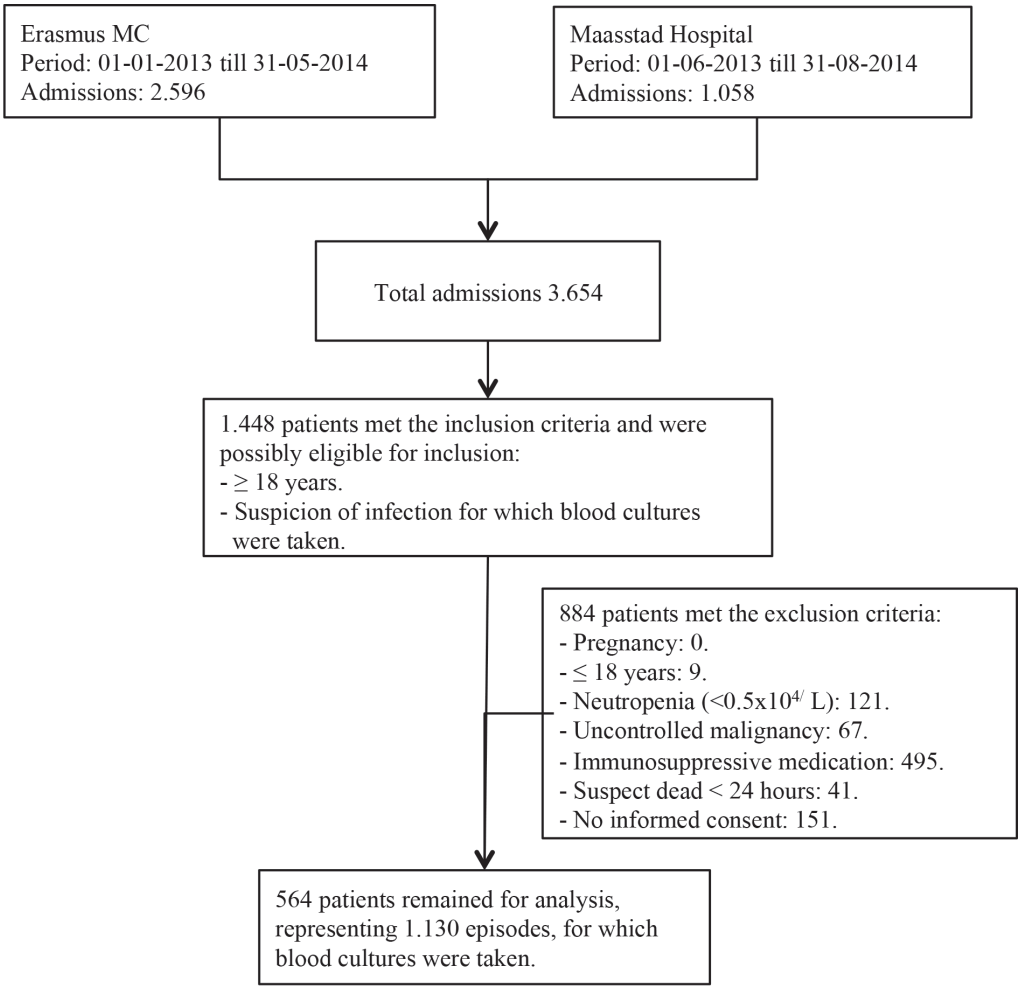
We performed a prospective, single-blinded, cluster-randomized, cross-over trial, involving the intensive care unit (ICU) of the Erasmus Medical Center Rotterdam and the Maasstad hospital Rotterdam. We conducted this trial between January 2013 and September 2014. The ICU of the Erasmus Medical Center is a tertiary care mixed medical-surgery ICU with 2000 admissions per year. The ICU of the Maasstad Hospital is a secondary care mixed medical-surgical ICU with 1200 annual admissions. The trial was conducted in accordance with the ethical principles decreed by the Declaration of Helsinki and in compliance

with International Conference on Harmonization Good Clinical Practice Guidelines. The final protocol, amendments and informed consent document were reviewed and approved by the institutional review board (IRB) or the independent medical ethical committee at each of the investigational centers. This study was finally approved by the medical ethical committee of the Erasmus Medical Center (MEC 2011-505) and registered with ClinicalTrial.gov (protocol ID NCT 01847079). All patients or their proxy provided written informed consent prior to study inclusion, as a presumed consent at ICU admission.

Patients on the intensive care unit above the age of 18 years in whom a suspicion of infection was raised and taking blood for culture was clinically indicated by the attending intensivist were enrolled in the study. Suspicion of infection could be increasing body temperature above 38.3° C (tympanic temperature), chills, progressive leukocytosis or increased C-reactive protein (CRP), increasing consolidations on chest radiography or other imaging of potential infection sources. Patients could be included more than once; every time that blood for culture is taken counts as a suspicion of infection episode (SIE). Patients were excluded if they had one of the following exclusion criteria: pregnancy, neutropenia (defined as leukocyte count less than $0.5x10^9$/L) and preterminal illness with an expected death within 24 hours. Patients were not included if blood cultures were performed as part of a standard protocol (such as patients with veno-venous or veno-arterial extracorporeal membrane oxygenation) or were performed to check the effectiveness of treatment (such as in endocarditis), unless the blood culture was done because of a SIE. A flow chart of the included patients is given in Additional file 1, Figure 1.

Patients were otherwise taken care of by board certified intensivists, according to local and national guidelines. In case of a microbial infection source control was performed when possible and antibiotic treatment was given in close collaboration with a medical microbiologist.

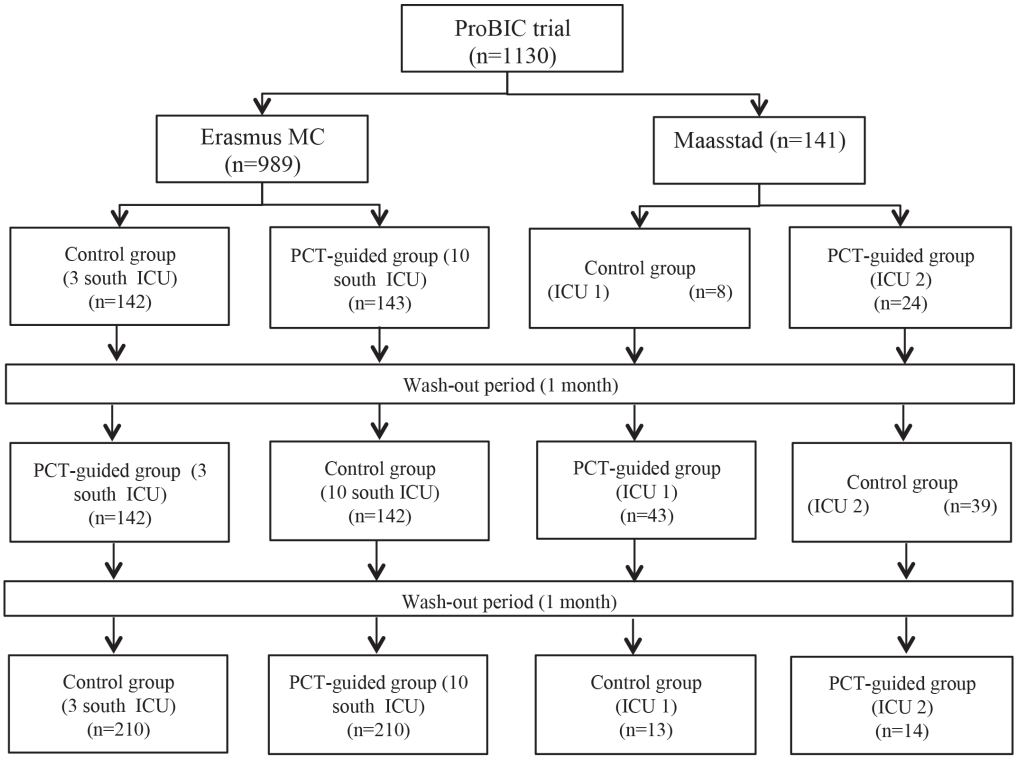
Study protocol. The participating ICU's (2 per medical center) were stratified and randomized by treatment regimen into a control group (standard of care) and a PCT-guided group. Randomization was performed per cluster allocation, being an assigned ICU. All patients included into this trial followed the regimen which was allocated to the ICU for that period. The participating units switched the allocated regimen every three months. We used a wash-out period between the cross-over period, to minimize the risk for a patient to follow two



Additional file 1: Figure 1. Flowchart of patients who met the inclusion and exclusion criteria

different regimens. The wash-out period was set for 1 month, in which >99% of the patients in the previous period has left the ICU. None of the patients included into this study followed two regimens. The participating ICU's were matched for a 1:1 ratio of allocation (Additional file 2, Figure 2).

In both the control and PCT-guided group blood was taken at the same moment for the PCT measurement and blood cultures. In the control group, 2 sets of blood cultures were sent directly to the department of medical microbiology. The PCT measurement in the control group was determined by the department of clinical chemistry, however the results of the measurement were blinded for the investigators and only available prior to analysis. In the PCT-



Additional file 2: Figure 2. Flowchart of the cluster allocation (BC = blood culture; ICU = intensive care unit; PCT = procalcitonin).

guided group the PCT measurement was determined as a stat determination, rendering results within 1 hour.

Blood samples for the PCT measurement were immediately centrifuged at 3000rpm for 10 minutes at room temperature (HETTICH Rotina 420R, Tuttlingen, Germany). The PCT measurement was performed on the automated Kryptor platform (BRAHMS AG, Hennigsdorf, Germany), using the Roche Elecsys BRAHMS PCT assay. Upon receiving results, the attending intensivists determined whether to send the blood cultures to the department of medical microbiology or not. We used a cut-off of 0.25 ng/ml in the PCT-guided group. Values below this cut-off were regarded as normal, and thus not worth culturing (and blood cultures taken were destroyed). It was possible for the attending intensivists to overrule the PCT-guided strategy and still send in blood cultures at normal PCT. Values higher than the cut-off of 0.25 ng/ml were regarded as a possible infection, and in these patients blood cultures were sent to the department of medical microbiology for further analysis. Each set of blood cultures

consists of one aerobic and one anaerobic bottle (BD Bactec™, New Jersey, USA) containing resin to enhance recovery of organisms. The blood cultures were incubated for 7 days in an automatic analyser (BD BACTEC™, New Jersey, USA) that automatically demonstrates the time to positive blood culture in case of positive bacterial or fungal growth. Gram strains were performed, and the organisms were cultured on agar plates and after growth identification was performed, using the VITEK® 2 (Biomerieux, Marcy l'Etoile, France). Polymerase chain reaction (PCR) technique was carried out using a LightCycler480 PCR-system (Roche Diagnostics, Almere, The Netherlands) to detect viral growth in blood samples. Bacteraemia was defined as having a positive blood culture with a recognized pathogen except skin contaminants [18,19]. In case of skin contaminants, bacteraemia was only considered if at least 2 blood cultures drawn on separate occasions were positive for the same microorganism [18,19]. We otherwise determined inflammatory parameters such as CRP and white blood cell counts.

Data collection

At the day of inclusion, baseline demographic data and clinical variables, including age, sex, pre-morbidity, reasons of admission, use of antibiotics including SDD, antifungal treatment, steroids, immunosuppressive medication, immune status (active malignancy or other causes of an immunocompromised state), recent surgery, mechanical ventilation, renal replacement therapy, total parenteral nutrition, central venous catheters and vital parameters were recorded. The acute physiology and chronic health evaluation IV (APACHE IV) and the sequential organ failure assessment (SOFA) score were recorded at admission. Patients were followed until day 28 and 90 after inclusion and length of ICU stay and vital outcomes were recorded.

Statistical analysis

The primary outcome measurement of this study is safety, expressed as mortality at day 28 and 90. We calculated that 550 patients were needed to determine non-inferiority in a parallel group design with a power of 90%, an one sided alpha error of 5% and a non-inferiority limit of 10% [20]. The sample size calculation was based on an assumed risk of death of 20% in both the control and intervention group [21-24]. A hierarchical poisson regression model, using the

logarithm of the survival time as an offset variable, was used to estimate the relative risk of mortality and associated 95% confidence intervals (CI) between control and PCT-guided groups. The ward and ward-period interaction were used as random effects to account for systematic effects of ward and period on the outcome [25]. The results were deemed non-inferior when the 95% CI was below 1.20 since our design was more complicated than a parallel group design and had precluded a power analysis based on poisson regression. We performed an intention-to-treat analysis (all PCT-guided vs control patients), per protocol analysis (PCT-guided patients without blood cultures vs controls), and an as-treated analysis (PCT-guided patients without blood cultures vs PCT-guided patients with blood cultures and controls). All analyses were performed using R version 3.2.1 and the hierarchical poisson models were fitted using the *lme4* package. We provide an overview with the involved costs and savings when using a PCT-guided strategy. Data are expressed as median (interquartile range) or as number of patients (percentage) where appropriate. Indeed, data were distributed non-normally (Kolmogorov-Smirnov test $P<0.05$). The Mann-Whitney U test and Fisher exact test were used to compare two groups. All tests were 2 sided, and $P<0.05$ was considered statistically significant. Exact *P* values > 0. 001 are given.

Results

Descriptives

In total, 1.448 patients were possibly eligible for inclusion, of whom 564 were included and remained for analysis (Figure 1). The control group consisted of 288 patients and the PCT-guided group of 276 patients (Table 1). No difference was observed between the groups, except for more neurologic premorbidity in the control group (Table 1).

Suspected infection episodes (SIE)

Table 2 shows that the control group represented 554 SIE against 576 SIE in the PCT-guided group, with a somewhat higher CRP in the latter. In the control group there were 118 episodes of bacteraemia in 58 patients, against 156 episodes in 63 patients in the PCT-guided group (Table 3). In only 6 episodes

Table 1. Baseline demographic and clinical characteristics.

	Control group (n=288)	PCT-guided group (n=276)	P
Age (years)	59 (21)	61 (22)	0.36
Gender (male)	200 (69)	177 (64)	0.21
APACHE IV score	62 (36)	67 (45)	0.27
SOFA score	8 (6)	8 (6)	0.79
Premorbidity			
Neurologic	75 (26)	47 (17)	0.02
Cardiac	95 (33)	91 (33)	0.99
Pulmonary	66 (23)	50 (18)	0.11
Gastro intestinal	89 (31)	91 (33)	0.60
Renal	37 (13)	28 (10)	0.26
DM II	55 (19)	47 (17)	0.45
Cancer	63 (22)	77 (28)	0.08
Auto immune	14 (5)	14 (5)	0.93
Steroids	46 (16)	50 (18)	0.57
Immune suppression	20 (7)	22 (8)	0.64
Reasons of ICU admission			0.70
Suspected sepsis	86 (30)	83 (30)	
Respiratory failure	54 (19)	61 (22)	
Renal failure	1 (1)	2 (1)	
Liver failure	0	2 (1)	
Neurology	35 (12)	26 (9)	
CPR	14 (5)	14 (5)	
Shock	12 (4)	7 (3)	
Trauma	17 (6)	14 (5)	
Postoperative	55 (19)	58 (21)	
Miscellaneous	14 (5)	9 (3)	
Treatment in ICU			
Mechanical ventilation	245 (85)	246 (89)	0.15
Renal Replacement Therapy	63 (22)	69 (25)	0.44
ECMO	9 (3)	3 (1)	0.09
CVC	210 (73)	179 (65)	0.04
Norepinephrine	225 (78)	232 (84)	0.07
Antibiotics	279 (97)	270 (98)	0.32
Total parenteral nutrition	86 (30)	99 (36)	0.13

Numbers (percentage) or median (interquartile range), where appropriate. Abbreviations: APACHE IV = Acute Physiology and Chronic Health Evaluation II; CPR = cardiopulmonary resuscitation; CVC = central venous catheter; DM II = diabetes mellitus type II; ECMO = extra corporeal membrane oxygenation; SOFA = sequential organ failure assessment score.

Table 2. Infection markers of all 1130 suspected infection episodes.

	Control group (n=554)	PCT-guided group (n=576)	P
Temperature (°C)	38.1 (1.6)	38.0 (1.6)	0.14
Heart rate (beats/ min)	109 (32)	111 (32)	0.11
Respiratory rate (breaths/ min)	30 (16)	28 (18)	0.97
WBC (10^9/ L)	12.6 (9.7)	12.5 (9.9)	0.40
CRP (mg/L)	111 (147)	148 (178)	<0.001
PCT (μg/ L)	1.1 (5.2)	1.4 (6.1)	0.18

Median (interquartile range). Abbreviations: CRP = C- reactive protein; PCT = procalcitonin; WBC = white blood cell count.

of bacteraemia the PCT value was below 0.25 ng/mL (3x *Enterococcus faecalis*, 1x *Enterococcus faecium*, 1x *Staphylococcus aureus* and 1x *Staphylococcus epidermidis*). The genus of the micro-organism cultures in the other 268 episodes were: 99x staphylococci, 39x streptococci, 88x enterobacteriaceae, 7x bacteroides, 5x bacillus, 2x burkholderia, 6x pseudomonas, 24x fungi and 4x viral.

Predictive values

A low <0.25 ng/mL PCT occurred in 121 and 76 SIE in the control and PCT-guided group, respectively (Table 3). The sensitivity of a low PCT for predicting bacteraemia was 98% with a specificity of 20%, negative predictive value 96% and positive predictive value 29%.

Primary outcome

No difference was seen in 28-day or 90-day mortality between the groups (Table 4). In the PCT-guided group, a PCT <0.25 ng/mL occurred in 37 patients. Applying the study protocol saved 18 sets of blood cultures in 17 patients with a low PCT. These 17 patients had a 28 and 90-day mortality rate of 29% and 35% respectively, whereas the other 20 patients with low PCT and blood cultures taken had a mortality rate of 19% and 29%, respectively. The intention to treat analysis showed a hazard ratio of 0.85 (95% CI, 0.62-1.17) and 0.89 (95% CI, 0.67-1.17) for 28- day and 90-day mortality, respectively, favoring the PCT-guided group. The per protocol analysis showed a hazard ratio of 0.89 (95% CI, 0.67-1.17) and 0.89 (95% CI, 0.67-1.17) for 28- day and 90-day mortality, respectively. The as-treated analysis showed a hazard ratio of 0.89 (95% CI, 0.67-1.17) and 0.89 (95% CI, 0.67-1.17) for 28-day and 90-day mortality, respectively. The results

Table 3. Procalcitonin measurements and blood culture results for the different SIE.

	SIE I		SIE II		SIE III and IV	
	Control group (n=288)	PCT-group (n=276)	Control group (n=149)	PCT-group (n=127)	Control group (n=117)	PCT-group (n=173)
Number (percentage) of blood cultures taken	288 (100)	259 (94)	149 (100)	126 (99)	117 (100)	173 (100)
Positive blood cultures	71 (25)	70 (25)	43 (29)	37 (29)	37 (32)	62 (36)
PCT < 0.25 ng/mL and BC negative	47 (16)	15 (5)	24 (16)	15 (12)	25 (21)	17 (10)
PCT < 0.25 ng/mL and BC contamination	8 (3)	4 (1)	6 (4)	2 (2)	7 (6)	3 (2)
PCT < 0.25 ng/mL and BC positive	2 (1)	1 (1)	1 (1)	1 (1)	1 (1)	0
PCT ≥ 0.25 ng/mL and BC negative	170 (59)	174 (63)	82 (55)	74 (58)	55 (47)	94 (54)
PCT ≥ 0.25 ng/mL and BC contamination	4 (1)	3 (1)	1 (1)	0	7 (6)	1 (1)
PCT ≥ 0.25 ng/mL and BC positive	57 (20)	62 (22)	35 (23)	34 (27)	22 (19)	58 (33)
On antibiotics						
PCT < 0.25 ng/mL and BC negative	43 (91)	30 (94)	24 (100)	16 (100)	25 (100)	17 (100)
PCT < 0.25 ng/mL and BC contamination	8 (100)	4 (100)	6 (100)	2 (100)	7 (100)	3 (100)
PCT < 0.25 ng/mL and BC positive	2 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0
PCT ≥ 0.25 ng/mL and BC negative	167 (98)	171 (98)	81 (99)	74 (100)	55 (100)	94 (100)
PCT ≥ 0.25 ng/mL and BC contamination	4 (100)	3 (100)	1 (100)	0	7 (100)	1 (100)
PCT ≥ 0.25 ng/mL and BC positive	57 (100)	62 (100)	35 (100)	34 (1000	22 (100)	58 (100)

Abbreviations: BC = blood culture; PCT = procalcitonin.

Table 4. Primary outcome measures for SIE I.

	Control group	PCT-guided group	P
	(n=288)	(n=276)	
Length of ICU stay (days)	10 (17)	12 (20)	0.21
Length of hospital stay (days)	23 (35)	31 (39)	0.005
Mortality day 28	92 (32)	80 (29)	0.36
Mortality day 90	115 (40)	105 (38)	0.53
PCT<0.25 and no BC sent in	**(n=0)**	**(n=17)**	
Mortality day 28	-	5 (29)	-
Mortality day 90	-	6 (35)	-
PCT<0.25 and BC sent in	**(n=57)**	**(n=20)**	
Mortality day 28	11 (19)	4 (19)	0.57
Mortality day 90	15 (26)	6 (29)	0.65
PCT>0.25 and BC sent in	**(n=231)**	**(n=239)**	
Mortality day 28	81 (35)	71 (30)	0.20
Mortality day 90	100 (43)	93 (39)	0.27

Abbreviations: BC = blood culture; PCT = procalcitonin.

Table 5. Potential cost analysis in 1130 suspected infection episodes.

If we applied the study protocol to all included patients and would have not taken a blood culture in all patients with procalcitonin (PCT) <0.25 ng/mL, 197 cultures would have been saved, while a blood culture costs €35.20. The extra costs involved are those of PCT for €5.00 per determination.

Extra costs PCT measurement	
1130 x €5.00	€5.650,00
Saved cultures	
197 x €35.20	€6.934,40 -
Total	- €1.284,40

were deemed non-inferior since the upper limit of the 95% CI was below the margin of 1.20. The length of ICU stay was comparable between groups, but the length of hospital stay was longer in the PCT-guided group (Table 4).

Secondary outcome

If in practice a low PCT would always be followed by withholding blood cultures it would save €1,284.40 (Table 5) per 1,130 SIE, or €1.14 per SIE .

Discussion

This study evaluated the predictive value of PCT for bacteraemia and the safety and cost-effectiveness of a normal PCT to withhold blood cultures in critically ill patients with a SIE. Based on the high negative predictive value and the regression analyses, which did not exceed a 20% group difference for mortality, a low PCT can be safely used to rule out bacteraemia. This could lead to a modest cost reduction when blood culturing is withheld or postponed.

This study suggests that a PCT-guided algorithm for performing blood cultures can be safely applied. Mortality rates in this study are comparable with the observed 28- day mortality rates of 20-38% found in a large systematic review and meta-analysis on PCT-guided antibiotic therapy in critically ill patients with bacteraemia [22]. The overall ICU mortality rate was 27% (30% control group vs 24% PCT-guided group) and the overall in hospital mortality rate was 35% (38% control group and 33% PCT-guided group) which agrees with an international study of the prevalence and outcomes of infection in ICU's [5]. There is no obvious explanation for a longer hospital length of stay in the PCT-guided group.

The observed occurence of bacteraemia in this study varies between 20-23% which is comparable with a recently performed meta-analysis on the diagnostic accuracy of PCT for bacteraemia with a described occurence of 21% [9]. In the current study we have chosen to use a cut-off value of 0.25 ng/mL for PCT. The 0.25 ng/ml cut-off was chosen based on two previous studies which looked at the predictive value of PCT for bacteraemia in patients on the emergency department [26, 27]. Both studies suggested that a PCT value ≤0.25 ng/ml can be used to rule out bacteraemia and suggested that using this cut-off could possibly be useful to save blood cultures in large volumes [26, 27]. With the used cut-off of 0.25 ng/ml we found a sensitivity of 98% and a specificity of 20% for the prediction of bacteraemia. Other studies performed on the diagnostic accuracy of PCT for bacteraemia in the critically ill used a cut-off value of 0.5 ng/ml [6, 10-17]. By using a cut-off of 0.5 ng/ml we found a sensitivity of 86% and specificity of 32% for the prediction of bacteraemia. The results are comparable with the other performed studies on the diagnostic accuracy of PCT for bacteraemia [6, 10-17]. The corresponding negative and positive predictive value at a cut-off value of 0.25 ng/ml are 96% and 29%, respectively, and are comparable with the observed negative predictive value of 98% and positive predictive value of 28% in a large meta-analysis [9]. With the used cut-off, PCT classified 143 SIE's correctly for not having bacteraemia (true negative) and classified 6 SIE's false for not having bacteraemia (false negative). In all cases, the cultured micro-organisms were susceptible for antibiotics and all patients were treated with vancomycin or flucloxacillin in case of the cultured *Staphylococcus epidermidis*. In all patients arterial and central venous catheters were replaced. The question remains if we would have missed cases of severe bacteraemia by using the PCT strategy and destruction of all blood cultures at low PCT and whether this would have harmed patients. Many experts favor deferring antimicrobial therapy for a bacteraemia caused by Enterococcus species in the setting of a single positive blood culture, since catheter removal alone may be sufficient enough to cure the infection in case the intravascular catheter is the likely source of bacteraemia [8,28,29]. In the 4 cases of enterococcal bacteraemia in this study only a single blood culture was positive and the 4 cases could be regarded as low grade infection in which removal of indwelling catheters could have been sufficient treatment. The other 2 cases should be regarded as true bacteraemia which would have been falsely classified as having no bacteraemia if cultures had been destroyed. In the case

of the *Staphylococcus epidermidis*, 3 sets of blood cultures were even positive. We may speculate that in the PCT-group with low PCT and discarded blood cultures, similar, low pathogenic microorganisms as in controls were involved. We may also speculate that PCT had risen in a second SIE, with positive blood cultures. Otherwise, clinical judgement should always predominate over PCT determinations.

A total of 1130 blood cultures were taken in this study of which 197 (17%) could have been saved by using PCT as a pre-test. The observed incidence of 17% is much lower compared with two other studies which predicted a possible reduction of 37-40% of blood cultures [26,27]. These studies were primarily designed to evaluate the predictive value of PCT for bacteraemia in patients in the emergency department and not to study the usefulness of PCT to guide efficient use of blood cultures. Both studies used a cut-off of 0.25 ng/mL and described sensitivities of 96% and 94%, respectively, which are comparable with our study [26,27]. The observed difference could be explained by the fact that both studies were performed on the emergency department or primary care unit, including patients with a clinical suspicion of urinary tract infection or pneumonia. In contrast to our study which was performed in critically ill patients, in which a suspected infection can be proven in only 50% or less. Despite the lower incidence of 17% in blood culture reduction, applying a PCT-guided strategy on the ICU can still be cost-effective, as our study suggests, moreover using this strategy results in lower volumes of blood taken from patients (10 mL for a PCT measurement versus 40 ml for a blood culture) and thereby a possible reduction of the risk of anemia caused by repetitive laboratory measurements [30].

This study has several limitations. First of all, in only 17 (46%) patients with low PCT levels, blood cultures were saved in the PCT-guided group. When the protocol was strictly followed, blood cultures could be saved in 76 SIE's, for which a classic intention to treat analysis could be performed. However, we used a hierarchical approach as described by Christiansen and Morris, having several advantages such as removal of regression to the mean bias and the use of smaller sample sizes in the analysis [25]. Second, for the primary sample size calculation of this study we used a non-inferiority limit of 10%. Since our study design was more complicated than a parallel group design and had precluded a power analysis based on poisson regression, we decided to use a non-inferiority limit of 20% for final analysis of the study. A non-inferiority limit is usually

set at 10-20%, however, choosing an non-inferiority limit remains difficult and debatable [31].

Conclusion

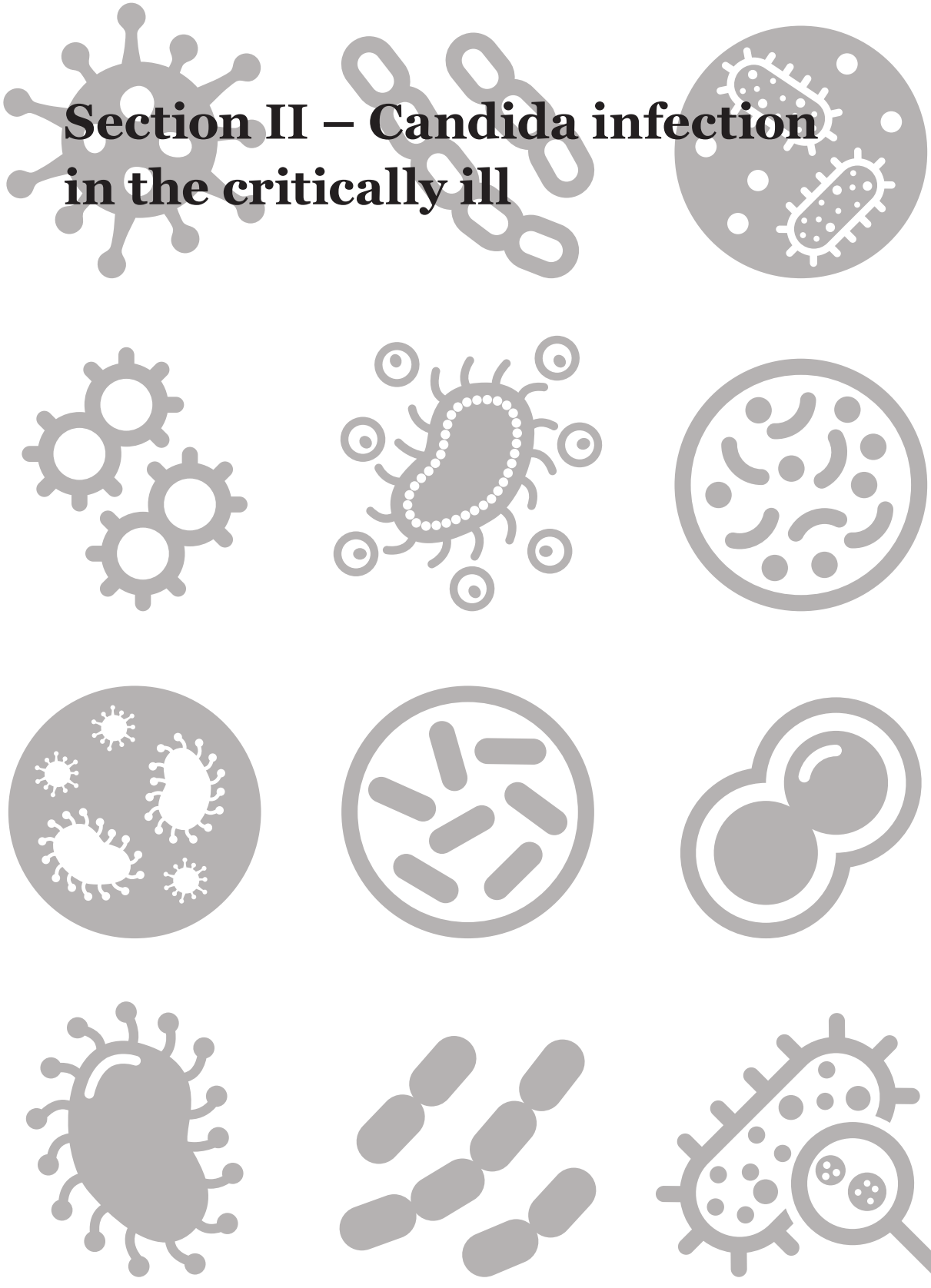
In conclusion, this prospective multicenter randomized trial showed that using a PCT-guided strategy to obtain blood cultures in critically ill patients with a suspicion of infection seems to be safe and (cost-)effective.

References

1. Mnatzaganian G, Galai N, Sprung CL, Zitser-Gurevich Y, Mandel M, Ben-Hur D, et al. Increased risk of bloodstream and urinary infections in intensive care unit (ICU) patients compared with patients fitting ICU admission criteria treated in regular wards. *J Hosp Infect* 2005; 59: 331-42.
2. Soogaard M, Norgaard M, Dethlefsen C, Schonheyder HC. Temporal changes in the incidence and 30-day mortality associated with bacteremia in hospitalized patients from 1992 through 2006: a population-based cohort study. *Clin Infect Dis* 2011; 52: 61-9.
3. Kumar A, Roberts D, Wood K, Light B, Parrillo J, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006; 34: 1589-1596.
4. Kibe S, Adams K, Barlow G. Diagnostic and prognostic biomarkers of sepsis in critical care. *J Antimicrob Chemother* 2011; 66: 33-40.
5. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in Intensive Care Units. *JAMA* 2009; 302: 2323-2329.
6. Hoeboer SH, Alberts E, Van den Hul I, Tacx AN, Debets-Ossenkopp YJ, Groeneveld AB. Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: a case for procalcitonin. *J Infect* 2012; 64: 484-493.
7. Vincent JL, Beumier M. Diagnostic and prognostic markers in sepsis. *Expert Rev Anti Infect Ther* 2013; 11: 265-275.
8. Hoenigl M, Raggam RB, Wagner J, Prueller F, Grisold AJ, Leitner E, et al. Procalcitonin fails to predict bacteremia in SIRS patients: a cohort study. *Int J Clin Pract* 2014; 68: 1278-1281.
9. Hoeboer SH, Van der Geest PJ, Nieboer D, Groeneveld AB. The diagnostic accuracy of procalcitonin for bacteraemia: a systematic review and meta-analysis. *Clin Microbiol Infect* 2015; 21: 1198-743.
10. Bell K, Wattie M, Byth K, Silvestrini R, Clark P, Stachowski E, et al. Procalcitonin: a marker of bacteraemia in SIRS. *Anaesth Int Care* 2003; 31: 629-636.
11. Bogar L, Molnar Z, Kenyeres P, Tarsoly P. Sedimentation characteristics of leucocytes can predict bacteraemia in critical care patients. *J Clin Pathol* 2006; 59: 523-525.
12. Charles PE, Ladoire S, Snauwaert A, Prin S, Aho S, Pechinot A, et al. Impact of previous sepsis on the accuracy of procalcitonin for the early diagnosis of blood stream infection in critically ill patients. *BMC Infect Dis* 2008; 8: 163.
13. Nakamura A, Wada H, Ikejiri M, Hatada T, Sakurai H, Matsushima Y, et al. Efficacy of procalcitonin in the early diagnosis of bacterial infections in a critical care unit. *Shock* 2009; 31: 586-591.
14. Karlsson S, Heikkinen M, Pettilä V, Alila S, Väisänen S, Pulkki K, et al. Predictive value of procalcitonin decrease in patients with severe sepsis: a prospective observational study. *Crit Care* 2010; 14: R205.
15. Theodorou VP, Papaioannou VE, Tripsianis GA, Panopoulou MK, Christophoridis EK, Kouliatsis GA, et al. Procalcitonin and procalcitonin kinetics for diagnosis and prognosis of intravascular catheter-related bloodstream infections in selected critically ill patients: a prospective observational study. *BMC Infect Dis* 2012; 12: 247.
16. Pereira JM, Teixeira-Pinto A, Basílio C, Sousa-Dias C, Mergulhão P, Paiva JA. Can we predict pneumococcal bacteremia in patients with severe community-acquired pneumonia? *J Crit Care* 2013; 28: 970-974.
17. Shi Y, DU B, Xu YC, Rui X, DU W, Wang Y. Early changes of procalcitonin predict bacteremia in patients with intensive care unit-acquired new fever. *Chin Med J* 2013; 126: 1832-1837.
18. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. *Am J Inf Control* 1988; 16: 128-140.

19. Calandra T, Cohen J. International sepsis forum definition of infection in the ICU consensus conference. *Crit Care Med* 2005; 33:1538-1548.
20. Sealed Envelope Ltd. 2012. Power calculator for binary outcome non-inferiority trial. [Online] Available from: https://www.sealedenvelope.com/power/binary-noninferior/ [accessed Monday Apr 25 2016].
21. Burkhardt O, Ewig S, Haagen U, Giersdorf S, Hartmann O, Wegscheider K, et al. Procalcitonin guidance and reduction of antibiotic use in acute respiratory tract infection. *Eur Respir J* 2010; 36: 601-607.
22. Kopterides P, Siempos, II, Tsangaris I, Tsantes A, Armaganidis A. Procalcitonin-guided algorithms of antibiotic therapy in the intensive care unit: a systematic review and meta-analysis of randomized controlled trials. *Crit Care Med* 2010; 38: 2229-2241.
23. Schuetz P, Albrich W, Christ-Crain M, Chastre J, Mueller B. Procalcitonin for guidance of antibiotic therapy. *Expert Rev Anti Infect Ther* 2010; 8: 575-587.
24. Long W, Deng X, Zhang Y, Lu G, Xie J, Tang J. Procalcitonin-guidance for reduction of antibiotic use in low-risk outpatients with community acquired pneumonia. *Respirology* 2011; 16: 519-24.
25. Christiansen CL, Morris CN. Hierarchical Poisson regression modeling. *J Am Stat Assoc* 1997; 92: 618-632.
26. Müller F, Christ-Crain M, Bregenzer T, Krause M, Zimmerli W, Mueller B, et al. Procalcitonin levels predict bacteremia in patients with community-acquired pneumonia: a prospective cohort trial. *Chest* 2010; 138: 121-129.
27. Van Nieuwkoop C, Bonten TN, Van 't Wout JW, Kuijper EJ, Groeneveld GH, Becker MJ, et al. Procalcitonin reflects bacteremia and bacterial load in urosepsis syndrome: a prospective observational study. *Crit Care* 2010; 14 R206.
28. Gullberg RM, Homann SR, Phair JP. Enterococcal bacteremia; analysis of 75 episodes. *Rev Infect Dis* 1989; 11: 74.
29. Hoge CW, Adams J, Buchanan B, Sears SD. Enterococcal bacteremia: to treat or not to treat, a reappraisal. *Rev Infect Dis* 1991; 13: 600-605.
30. Smoller BR, Kruskall MS. Phlebotomy for diagnostic laboratory tests in adults. Pattern of use and effect on transfusion requirements. *N Engl J Med* 1986; 314: 1233-1235.
31. D'Agostino RB, Massaro JM and Sullivan LM. Non-inferiority trials: design concepts and issues – the encounters of academic consultants in statistics. *Statist Med* 2003; 22: 169-186.

Section II – Candida infection in the critically ill



Chapter 6

Safety and efficacy of amphotericin-B deoxycholate inhalation in critically ill patients with respiratory *Candida* spp. colonization: a retrospective analysis

Patrick J. van der Geest,[1] Erik I. Dieters,[1] Bart Rijnders,[2] and A.B. Johan Groeneveld.[1]

[1]Department of Intensive Care medicine, Erasmus MC, Rotterdam, the Netherlands.
[2]Department of Medical Microbiology, Erasmus MC, Rotterdam, the Netherlands.

BMC Infectious Diseases 2014; 14: 575.

Abstract

Purpose

To assess the safety and efficacy of amphotericin-B deoxycholate (ABDC) inhalation for the treatment of respiratory *Candida* spp. colonization in critically ill patients.

Methods

All non-neutropenic patients admitted into the intensive care unit (ICU) of a university hospital from December 2010 - 2011, who had positive Candida spp. cultures of the respiratory tract for more than 1 day and required mechanical ventilation >48 h were retrospectively included. The decision to start ABDC inhalation had been made by attending intensivists on clinical grounds in the context of selective decontamination of the digestive tract. Infection characteristics and patient courses were assessed.

Results

Hundred and thirteen consecutive patients were studied. Fifty-one of them received ABDC inhalation and their characteristics at baseline and day 1 of respiratory colonization did not differ from those of colonized patients not receiving treatment (n=62). The ABDC-treated group had a similar *Candida* spp. load but did not decolonize more rapidly as compared to untreated patients. The clinical pulmonary infection and lung injury scores did not decrease as in the untreated group. In a Cox proportional hazard model, the duration of mechanical ventilation was increased ($P<0.003$) by ABDC treatment independently of other potential determinants and *Candida* spp. colonization. No differences in ventilator-associated pneumonia or in overall mortality (up to day 90) were observed.

Conclusion

Treatment of respiratory *Candida* spp. colonization in non-neutropenic critically ill patients by inhaled ABDC may not affect respiratory colonization but may increase duration of mechanical ventilation, because of direct toxicity of the drug on the lung.

Introduction

In critically ill patients, *Candida* spp. are frequently cultured from non-sterile body sites. However, the clinical significance hereof is not easy to define. When *Candida* spp. are isolated from the respiratory tract, discriminating between relatively harmless colonization or invasive infection is still surrounded by controversy and leads to therapeutic dilemmas [1-8]. On the one hand, respiratory isolation of *Candida* spp. could reflect the patient's state of immunoparalysis and the elimination of normal flora through previous antibiotic treatment but may otherwise be relatively harmless. On the other hand, it could reflect a risk factor for invasive *Candida* spp. infections, even in non-neutropenic patients, such as candidemia or pulmonary candidiasis, a rare and difficult to diagnose deep infection in the critically ill [2,3]. Respiratory *Candida* spp. colonization has also been suggested to be a risk factor for (multidrug-resistant) Gram-negative airway infection, i.e. ventilator-associated pneumonia (VAP) or tracheobronchitis, to prolong ventilator dependency and to increase mortality, even in the absence of direct pulmonary pathogenicity [6,9-11].

One way to examine the clinical role of *Candida* spp. colonization of the respiratory tract is to examine the effect of treatment aimed at eradicating the fungus. The criteria to start antifungal drugs continue to be debated [1,4]. Nevertheless, one study suggests that systemic antifungal treatment may help to prevent *Pseudomonas* spp.-associated VAP in the critically ill patient colonized by *Candida* spp. in the respiratory tract [11]. Also, in neutropenic hematology patients at risk for invasive fungal infections inhalation therapy with liposomal amphotericin B has been proven to prevent invasive pulmonary aspergillosis [12,13]. ABDC inhalation is also used to prevent pulmonary aspergillosis in many lung transplant units but the safety and efficacy of this approach has never been validated in a large randomized placebo-controlled trial.

Selective decontamination of the digestive tract (SDD) is a strategy used in many Dutch intensive care units (ICU's) and consists of oral administration of non-absorbable antibiotics. When *Candida* spp. respiratory colonization is documented during routine surveillance cultures of the respiratory tract and the patient is at risk for deep infection, intensivists often start with amphotericin-B deoxycholate (ABDC) inhalation therapy via a nebulizer. This treatment is started as part of many SDD protocols used in large trials [14,15]. However, its

safety and efficacy are not well established, in the absence of randomized trials [16]. In rats, aerolized ABDC decreases activity of surfactant suggesting a possibility to harm [17-19]. In humans, however, (liposomal) amphotericin-B inhalation may not alter surfactant but may be cytotoxic by other means [20,21].

To further elucidate the significance of *Candida* spp. in respiratory secretions and the safety and efficacy of ABDC inhalation therapy, we retrospectively compared clinical courses of mechanically ventilated patients colonized with *Candida* spp. in the respiratory tract which received ABDC inhalation with those of a comparable group of patients in which ABDC was not initiated. The hypothesis was that ABDC is a safe and effective treatment for *Candida* spp. colonization of the respiratory tract and thereby prevents VAP and prolonged need for mechanical ventilation during SDD in the ICU.

Patients and methods

Patients

Data were retrospectively collected from our patient data management system, according to a predefined checklist. All patients >18 years of age requiring mechanical ventilation >48 h in the ICU of the Erasmus Medical Center Rotterdam, with at least 2 consecutive daily positive *Candida* spp. cultures of the respiratory tract (throat, tracheal aspirates or bronchoalveolar lavage), defining respiratory colonization, were included when admitted between December 2010 and December 2011. The ICU is a tertiary care mixed medical-surgical ICU with 2000 admissions per year. Patients with neutropenia (leukocyte count <0.5 $x10^9$/L), positive blood cultures for *Candida* spp., immunodeficiency (HIV, solid cancer, hematologic malignancy, solid- organ or bone marrow transplantation, or long term or high dose steroid treatment) or who did not meet respiratory colonization criteria were excluded (Figure 1). Informed consent was not needed according to the Dutch law because of the retrospective analysis in which data collected during routine clinical care were used and anonymously treated.

Clinical protocol

Patients requiring mechanical ventilation for more than 48 h receive SDD in our unit. This involves administration of an oral paste, a suspension via the

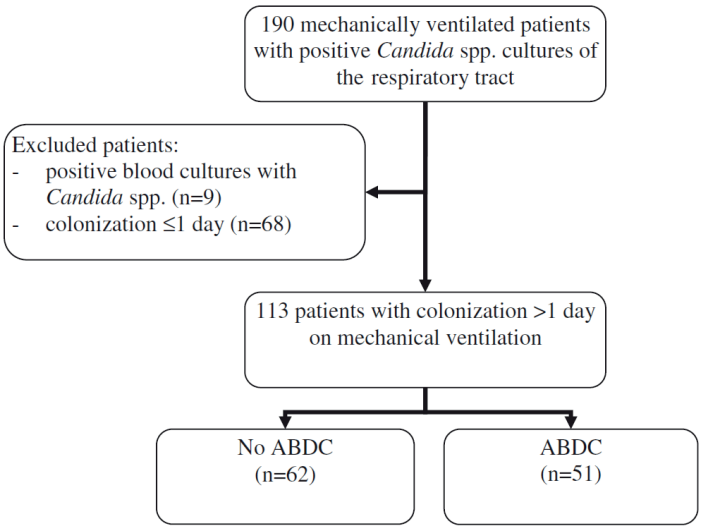


Figure 1. Patient inclusion. ABDC = amphotericin-B deoxycholate.

nasogastric tube and a suppository, containing the non- absorbable antibiotics tobramycin, amphotericin B and colistin. For the first three days, patients also receive cefotaxim intravenously at 4 times 1 gram a day. At the start of SDD, cultures are taken (inventory cultures) from throat, tracheal aspirates, urine, rectum and drains or wounds. To monitor the effect of the SDD, surveillance cultures (from throat, tracheal aspirates, urine, and rectum) are done routinely three times per week. SDD and surveillance cultures are continued until ICU death or discharge. On indication, additional cultures of the respiratory tract are taken at the discretion of the attending intensivist. Standard microbiological methods were used to culture the specimens. For the purpose of fungal detection in respiratory samples a Sabouraud agar (SB), incubated at 26°C was used. A Gram strain was performed to identify epithelial cells and bacteria. If the Gram strain contained 10 times more leukocytes than epithelial cells and if there were more than 6 species of one type of microorganism the streaked agar plates were incubated. *Candida* colonies are visually identified on the Sabouraud agar and identified to the species level using CHROMagar™. ABDC inhalation therapy is not part of the standard of care SDD regimen but can be added at the discretion of the attending intensivist when tracheal aspirates or throat cultures are repeatedly positive for *Candida* spp. and the patient is considered to be at risk for deep infection. For this purpose ABDC, dissolved in dextrose 5% with a final concentration of 10 mg/ mL is nebulized in a Maquet nebulizing system (Maquet Servo

Ultra Nebulizer, Maquet, Sweden), 4 times daily (daily dose 4x 4 mL = 40 mg). ABDC inhalation therapy is discontinued after two consecutive daily respiratory tract cultures or after detubation of the patient. Other antifungal therapy was instituted at the discretion of the attending intensivist based on cultures taken from other body sites. Pneumonia was diagnosed and treated by the attending intensivist on the basis of clinical, imaging and microbiological data in close collaboration with the infectious disease specialists. VAP was defined as the presence of a new or progressive pulmonary infiltrate on the chest radiograph along with infectious signs such as fever ≥38.5 °C or ≤36.5 °C, leukocyte count ≥10 x10^9/L or ≤4 x10^9/L and purulent tracheobronchial secretions [22].

Study protocol and data collection

At admission/baseline, demographic data and clinical variables, including age, sex, pre-morbidity, prior use of antibiotics including SDD and other risk factors for *Candida* spp. colonization, antifungal treatment, steroids, immune status (active malignancy or other causes of an immunocompromised state), reasons for admission, invasive procedures (arterial or venous central catheter, pulmonary artery catheter, endotracheal intubation) and treatment of organ failure (inotropic support, hemodialysis and mechanical ventilation settings) were recorded (but not all data are shown when considered not contribute). The simplified acute physiology score (SAPS II) was computed at admission. Day 1 of the study was defined as the first day of respiratory colonization with *Candida* spp. The acute physiology and chronic health evaluation II (APACHE II) and the sequential organ failure assessment (SOFA) were recorded at day 1. All cultures from the respiratory tract were recorded from day 1 until day of decolonization, i.e. the duration of respiratory colonization. Airway *Candida* decolonization was defined as the first day of 2 consecutive daily respiratory cultures negative for *Candida* spp. in the ICU. *Candida* spp. load was calculated as the number of positive respiratory cultures and the sum of the load (in thousands of colonies) of the cultures divided by the total cultures taken, using cultures from day 1 until decolonization, ICU death or discharge, whatever came first. *Candida* spp. load was classified as mild, <10^4, moderate, 10^4-10^5, and severe, >10^5. For the ABDC-treated group, the duration of respiratory colonization and load were calculated from day 1 until start of ABDC and from the start of ABDC until decolonization, ICU death or discharge, whatever came first. In order to calculate the clinical

pulmonary infection score (CPIS) [23] at day 1 and 7, routinely obtained temperature (°C), white blood cell count (WBC, 10^9/L), amount of tracheal secretions, chest radiography imaging results and P_aO_2/F_IO_2 ratio (kPa) were recorded using the worse data recorded during the 24 hours of that day. At day 1 and 7, we also recorded positive end-expiratory pressure (PEEP, cm H_2O), ratio of arterial PO_2 to inspiratory O_2 fraction (P_aO_2/F_IO_2, kPa), total respiratory dynamic compliance (mL/cm H_2O) and chest radiography imaging results to calculate the lung injury score (LIS) [24]. The compliance is calculated from tidal volume and difference between in- and expiratory pressures on the ventilator. The LIS is calculated by using the worst physical data recorded during the 24 hours of that day and the number of quadrants (0-4) on the chest radiograph showing alveolar consolidations. Patients were followed until day 28 and 90 after day 1 and duration of ICU stay, duration of colonization and mechanical ventilation (from day 1 until start of inhalational ABDC and thereafter until detubation not requiring reintubation within 48 h) and vital outcomes were recorded.

Statistical analysis

Continuous variables were expressed as the mean with standard deviation (SD) or when the assumption of normality (Kolmogorov-Smirnov test $P<0.05$) was violated as median values and interquartile ranges. Since ABDC treatment was started at median 5 days of colonization, patients colonized for 5 days or more were selected from the non-treated group for comparisons of durations. Kaplan-Meier curves were constructed and log rank testing was performed to evaluate group differences in colonization and ventilation durations, censored for death or discharge from the ICU. Multiple Cox proportional hazard modeling was done to evaluate the effect of ABDC treatment, irrespective of other variables associated with mechanical ventilation. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated. All tests were two-sided and $P<0.05$ was considered statistically significant. Exact P values >0.001 are given.

Results

Characteristics of patients who received ABDC (n=51) and those who did not (n=62) are summarized in Table 1. There tended to be more males in the treated

group, but groups were otherwise comparable at baseline up to day 1, suggesting similar general risks for respiratory *Candida* spp. colonization. However,

Table 1. Patient characteristics at admission, day 1 of colonization and clinical course.

	ABDC no	ABDC yes	P
	n=62	n=51	
Age, years	57 (17)	58 (20)	0.62
Gender, male	34 (55)	37 (73)	0.05
Premorbidity			
Cardiac	31 (50)	18 (35)	0.12
Gastointestinal	21 (34)	24 (47)	0.16
Cancer	17 (27)	16 (31)	0.65
Pulmonary	16 (26)	13 (25)	0.97
Neurological	11 (18)	12 (24)	0.45
DM	12 (19)	8 (16)	0.61
Renal	5 (8)	9 (18)	0.14
Immune	2 (3)	0 (0)	0.16
Other	20 (32)	14 (27)	0.58
Reasons of ICU admission			0.28
Suspected infection	18 (29)	18 (35)	
Respiratory failure	9 (15)	13 (25)	
Shock	9 (15)	6 (12)	
Neurological	11 (18)	4 (8)	
Medical	3 (5)	3 (6)	
Postoperative	6 (9)	4 (8)	
CPR	4 (6)	1 (2)	
Trauma	2 (3)	2 (4)	
SAPS II	43 (18)	48 (16)	0.12
At day 1 of colonization			
APACHE II	21 (12)	20 (9)	0.21
SOFA	9 (6)	9 (5)	0.18
Days from admission ICU	1 (3)	1 (2)	0.64
Ventilation from admission, days	1 (2)	1 (3)	0.18
Course			
Duration of stay ICU, days	10 (12)	24 (18)	<0.001
28-day mortality	19 (32)	10 (20)	0.13
90-day mortality	27 (45)	19 (37)	0.40

Numbers (percentage) or median (interquartile range), where appropriate. Abbreviations: ABDC = amphotericin-B deoxycholate; DM = diabetes mellitus; ICU = intensive care unit; CPR = cardiac pulmonary resuscitation; SAPS II = simplified acute physiology score; APACHE II = acute physiology and chronic health evaluation II; SOFA = sequential organ failure assessment score.

ABDC-treated patients had a longer duration of stay in the ICU but mortality did not differ from that in untreated patients.

Respiratory *Candida* spp. colonization, risk factors, bacterial infection and treatment characteristics according to ABDC inhalation treatment

Table 2 gives the data from day 1 of respiratory colonization until ICU death or discharge for the untreated and treated groups. Colonization lasted 5 (9) days in the untreated group and ABDC was started 5 (5) days after the day of first positive respiratory *Candida* spp. culture, so that we also compared groups for these comparable time frames. The data suggest similar risk and load of respiratory colonization with *Candida* spp. until treatment is started for both groups. However, the ABDC-treated group seemed to have an overall higher load and prolonged colonization than the untreated group, because of start of early and spontaneous decolonization in the latter. The CPIS score was higher 1 week after day 1 in the absence of more VAP in treated patients. Conversely, there was no decrease in the occurrence of VAP in the treated group. Even though

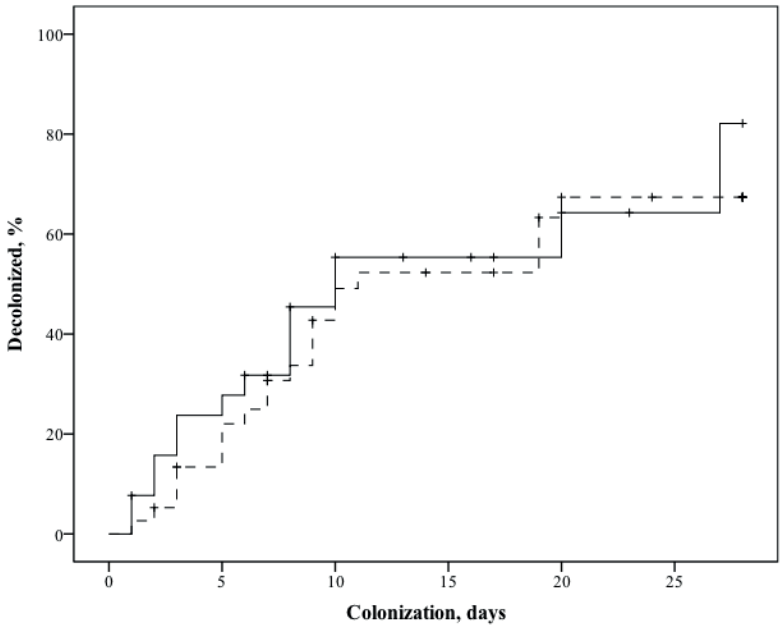


Figure 2. Duration of respiratory colonization from day 5 (now day 0 in graph) of colonization in untreated (continuous line) and from start of amphotericin B (ABDC) on day 0 in graph in treated patients (dotted line) until negative cultures, censored for death or discharge from the intensive care unit, to day 28; P = 0.45 (log-rank test).

Table 2. Respiratory Candida spp. colonization, risk factors, bacterial infection and treatment characteristics starting day 1 of colonization in untreated group and from day 1 of colonization to start of treatment in treated group and thereafter, until ICU death or discharge.

	ABDC no Day 1 until ICU death or discharge, n=62	ABDC yes Day 1 to start treatment, n=51	P vs no	ABDC yes Day 1 until ICU death or discharge, n=51	P vs no
Total *Candida* spp. cultures respiratory tract	2 (1)	3 (1)	0.67	4 (3)	<0.001
Candida spp. load			0.46		0.36
Mild, $<10^4$	38 (61)	32 (58)		30 (60)	
Moderate, 10^4-10^5	13 (21)	18 (33)		16 (32)	
Heavy, $>10^5$	11 (18)	5 (9)		4 (8)	
Candida albicans	41 (66)	34 (61)	0.45	32 (63)	0.74
Candida non-albicans	3 (4)	1 (2)		3 (6)	
Both *Candida* spp.	18 (30)	20 (37)		16 (31)	
Duration of respiratory colonization, days	5 (9)	5 (5)	0.66	12 (18)	<0.001
Corticosteroids	23 (37)	18 (35)	0.85	22 (43)	0.52
TPN	21 (34)	16 (31)	0.78	18 (35)	0.88
Ventilator-associated pneumonia,	23 (37)	11 (22)	0.16	18 (35)	0.96
Days after first *Candida* spp. culture	4 (6)	3 (4)	0.43	5 (4)	0.91
Gram positive	6 (10)	0 (0)		2 (4)	
Gram negative	17 (27)	11 (22)		16 (31)	
CPIS, day 1	7 (3)	8 (2)	0.10	NA	NA
day 7	5 (4)	NA	NA	8 (3)	<0.001
Systemic antifungal treatment	17 (27)	13 (26)	0.39	18 (35)	0.93
Echinocandins	11 (17)	9 (18)		11 (21)	
Azoles	6 (10)	4 (8)		7 (14)	

Numbers (percentage) or median (interquartile range), where appropriate. Abbreviations: ABDC = amphotericin-B deoxycholate; TPN = total parenteral nutrition; CPIS = clinical pulmonary infection score; day 1 and 7 refer to day of first isolation of *Candida* spp. in respiratory secretions and 1 week later, respectively; NA = not applicable.

total *Candida* spp. cultures in the respiratory tract after start of ABDC (1 (2)) was lower than before start (3 (1)) (P<0.001), respiratory colonization durations were similar, from day 5 (untreated group) or start of treatment (ABDC-treated group) to 28 days later (Fig. 2).

Patient courses

Table 3 gives the ventilation characteristics for both groups. The LIS and P_aO_2/F_IO_2 ratio at day 1 were similar between the groups, but at day 7 the LIS was higher and the P_aO_2/F_IO_2 ratio was lower in the ABDC-treated than untreated group. The PEEP was higher in the ABDC-treated group at day 7. Patients receiv-

Table 3. Ventilation characteristics at day 1 and 7 of colonization.

	ABDC no	ABDC yes	P
	n=62	n=51	
Tidal volume, ml/kg, day 1	7 (2)	7 (2)	0.77
Tidal volume, ml/kg, day 7	7 (3)	8 (3)	0.50
PEEP, cm H_2O, day 1	9 (4)	9 (4)	0.10
PEEP, cm H_2O, day 7	6 (5)	10 (6)	0.004
Compliance, mL/cm H_2O, day 1	37 (28)	44 (39)	0.05
Compliance, mL/cm H_2O, day 7	40 (27)	41 (36)	0.09
P_aO_2/ F_IO_2 ratio, day 1	213 (122)	197 (137)	0.40
P_aO_2/ F_IO_2 ratio, day 7	282 (123)	217 (147)	0.005
Chest X-ray, no. of quadrants, day 1	2 (2)	2 (1)	0.27
Chest X-ray, no. of quadrants, day 7	2 (3)	2 (2)	0.41
LIS, day 1	2.0 (1.0)	1.8 (1.3)	0.47
LIS, day 7	1.0 (1.3)	1.8 (1.5)	0.002
Ventilation after day 1, days	7 (9)	20 (16)	<0.001

Numbers (percentage) or median (interquartile range), where appropriate. Abbreviations: ABDC = amphotericin B deoxycholate; PEEP = positive end-expiratory pressure; P_aO_2 = partial pressure of oxygen in the blood (kPa); F_IO_2 = the percentage of oxygen administered; LIS = lung injury score; day 1 and 7 refer to day of first isolation of *Candida* spp. in respiratory secretions and 1 week later, respectively.

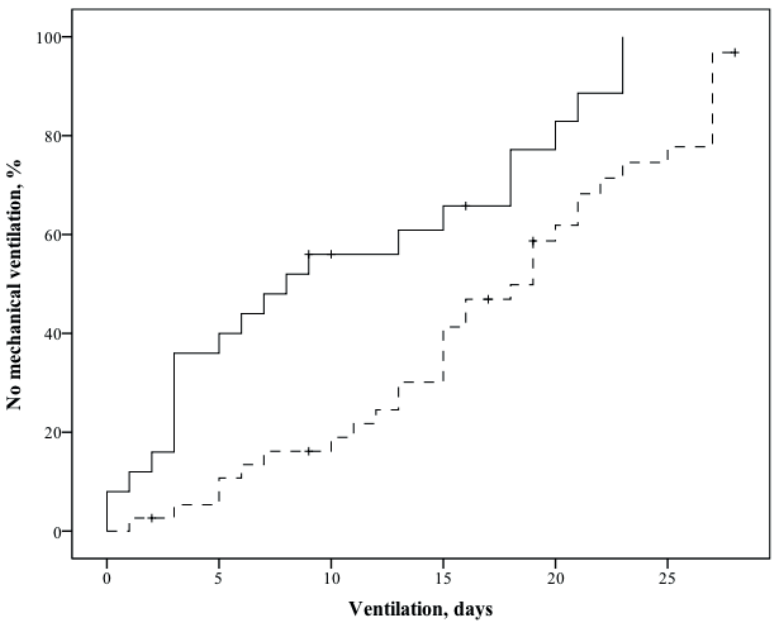


Figure 3. Duration of mechanical ventilation from day 5 (now day 0 in graph) of colonization in untreated (continuous line) and from start of amphotericin B (ABDC) on day 0 in graph in treated patients (dotted line), censored for death or discharge from the intensive care unit, to day 28; P = 0.003 (log-rank test).

ing ABDC were mechanically ventilated longer than patients without treatment (Figure 3), from day 5 or start of treatment onwards, in untreated and treated groups, respectively. The effect of ABDC on mechanical ventilation duration was independent from other factors such as duration of colonization after day 5 and LIS on day 7 (Table 4).

Table 4. Multiple Cox proportional hazard modeling for duration of mechanical ventilation.

	HR (95% CI)	P
ABDC treatment	3.63 (1.57-8.34)	0.003
Candida spp. load until day 5	1.19 (0.70-2.02)	0.51
Candida spp. load after day 5	0.67 (0.31-1.47)	0.32
Duration of colonization until day 5	0.96 (0.90-1.02)	0.13
Duration of colonization after day 5	0.92 (0.88-0.96)	<0.001
CPIS day 7	0.91 (0.76-1.10)	0.32
LIS day 7	0.60 (0.40-0.91)	0.02

Hazard ratios (HR; 95% confidence interval, CI) are given for each variable. Abbreviations: ABDC = amphotericin B deoxycholate; CPIS = clinical pulmonary infection score; LIS = lung injury score.

Discussion

This non-randomized observational study suggests that ABDC inhalation by critically ill, mechanically ventilated patients with respiratory *Candida* spp. colonization is not effective and potentially harmful. Patients on ABDC inhalation did not decolonize more rapidly, remained mechanically ventilated for an additional 13 days and had a longer ICU stay but no increased mortality, as compared to untreated patients.

Our study suffers from a non-randomised design, so that conclusions should be drawn carefully. Nevertheless, the data suggest that prior to start of treatment, disease severity, colonization risk and load were similar among the groups. Treatment was started approximately 5 days after the first positive respiratory *Candida* spp. culture, when in the untreated group spontaneous decolonization had already started. We are therefore uncertain, in disagreement with previous studies [16], whether decolonization would have also occurred in the treated group if treatment had been disadvised or postponed. However, when groups were matched for the same duration of respiratory colonization prior to treatment, no difference could be observed in rate of decolonization

(Figure 2), in contrast to the increase in decolonization with treatment from 62 to 86% observed in a previous, but larger, non-randomized study [16].

A previous study showed an incidence of VAP in patients with respiratory *Candida* spp. colonization of 24% which is comparable to the 29% incidence in this study [9]. However, our data do not suggest that *Candida* spp. colonization in the respiratory tract predisposed to VAP, in contrast to studies suggesting that this could promote VAP development, especially when caused by *Pseudomonas aeruginosa* and multidrug resistant bacteria [9,10]. Conversely, there is no suggestion that respiratory decolonization was associated with less VAP, in contrast to suggestions that systemic antifungal treatment decreases the incidence of VAP [11]. The differences in ventilation durations cannot be attributed to differences in the occurrence of VAP, even though the relatively non-specific CPIS (as well as the LIS) was higher on day 7 in the ABDC-treated group, since the CPIS was not a determinant of mechanical ventilation duration in multivariate analysis.

Safety issues of ABDC inhalation therapy remain a concern [19,20]. We therefore looked at pulmonary injury and ventilation durations, which appeared independent of respiratory colonization duration in our patients. The lung scores suggest greater injury in treated patients at day 7 already than in untreated patients, independent of VAP. Two studies showed surfactant dysfunction by ABDC, thereby deteriorating gas exchange [17,18], possibly via direct inhibition or damage of the alveolar capillary membrane resulting in an influx of surfactant-inactivating plasma proteins. Loss of surfactant could lead to longer requirement of mechanical ventilation in patients receiving inhalational ABDC. Indeed, the longer duration of mechanical ventilation and thus ICU stay in our study in ABDC-treated patients can be explained by pulmonary adverse effects of the drug. Our data do not exclude that ABDC inhalation prevents pulmonary infections with *Aspergillus* spp. but suggests that it may carry pulmonary toxicity.

In conclusion, treatment of pulmonary *Candida* spp. colonization in non-neutropenic critically ill patients by inhaled ABDC may not facilitate respiratory decolonization but may increase duration of mechanical ventilation, because of direct pulmonary toxicity of the drug. Therefore, inhalation of ABDC for respiratory colonization with *Candida* spp. in non-neutropenic critically ill patients cannot be recommended outside prospective randomized trials. For the latter, the use of less toxic liposomal dissolved formulation may be preferred [21,25].

References

1. El-Ebiary M, Torres A, Fabregas N, Puig de la Bellacasan J, Gonzalez J, Ramirez J, del Bano D, Hernandez C and Jimenez de Anta MT. Significance of the isolation of candida species from the respiratory samples in critically ill, non-neutropenic patients: an immediate post- mortem histologic study. Am J Respir Crit Care Med 1997; 156: 583-590.
2. Rello J, Esandi ME, Diaz E, Mariscal D, Gallego M and Valles J. The role of Candida sp isolated from bronchoscopic samples in nonneutropenic patients. Chest 1998; 14:146-149.
3. Charles PE, Dalle F, Doise JM, Quenot JP, Aho LS, Chavanet P and Blettery B. Candida spp. colonization significance in critically ill medical patients; a prospective study. Intensive Care Med 2005; 31: 393-400.
4. Eggimann P, Calandra T, Fluckiger U, Bille J, Garbino J, Glauser MP, Marchetti O, Ruef C, Tauber M and Pittet D. Invasive candidiasis: comparison of management choices by infectious disease and critical care specialists. Intensive Care Med 2005; 31: 1514-1521.
5. León C, Ruiz-Santana S, Saavedra P, Almirante B, Nolla-Salas J, Alvarez-Lerma F, Garnacho-Montero J and Leon MA. A bedside scoring system ("Candida score") for early antifungal treatment in non-neutropenic critically ill patients with Candida colonization. Crit Care Med 2006; 34: 730-737.
6. Delisle M-S, Williamson DR, Perreault MM, Albert M, Jiang X, Heyland DK. The clinical significance of Candida colonization of respiratory tract secretions in critically ill patients. J Crit Care 2008; 23: 11-17.
7. Meersseman W, Lagrou K, Spriet I, Maertens J, Verbeken E, Peetermans WE and Van Wijngaarden E. Significance of the isolation of Candida species from airway samples in critically ill patients: a prospective, autopsy study. Intensive Care Med 2009; 35: 1526-1531.
8. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, Meersseman W, Akova M, Arendrup MC, Arikan-Akdagli S, Bille J, Castagnola E, Cuenca-Estrella M, Donnelly JP, Groll AH, Herbrecht R, Hope WW, Jensen HE, Lass-Flörl C, Petrikkos G, Richardson MD, Roilides E, Verweij PE, Viscoli C and Ullmann AJ. ESCMID guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. Clin Microbial Infect 2012; 18: 19-37.
9. Azoulay E, Timsit JF, Tafflet M, de Lassence A, Darmon M, Zahar JR, Adrie C, Garrouste-Orgeas M, Cohen Y, Mourvillier B and Schlemmer B. Candida colonization of the respiratory tract and subsequent Pseudomonas ventilator- associated pneumonia. Chest 2006; 129: 110-117.
10. Hamet M, Pavon A, Dalle F, Pechinot A, Prin S, Quenot JP and Charles PE. Candida spp. airway colonization could promote antibiotic resistant bacteria selection in patients with suspected ventilator associated pneumonia. Intensive Care Med 2012; l38: 1272-1279.
11. Nseir S, Jozefowicz E, Cavestri B, Senid B, Di Pompeo C, Dewayrin F, Favory R, Roussel-Delvallez M and Durocher A. Impact of antifungal treatment on Candida-Pseudomonas interaction: a preliminary retrospective case-control study. Intensive Care Med 2007; 33: 137-142.
12. Rijnders BJ, Cornelissen JJ, Slobbe L, Becker MJ, Doorduijn JK, Hop WC, Ruijgrok EJ, Lowenberg B, Vulto A, Lugtenburg PJ and de Marie S. Aerolized liposomal amphotericin B for the prevention of invasive pulmonary aspergillosis during prolonged neutropenia: a randomized placebo controlled trial. Clin Infect Dis 2008; 46: 1401-8.
13. Kuiper L, Ruijgrok EJ. A review on the clinical use of inhaled amphotericin B. J Aerosol Med Pulm Drug Del 2009; 22: 213-227.
14. De Jonge E, Schultz MJ, Spanjaard L, Bossuyt PMM, Vroom MB, Dankert J and Kesecioglu J. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. The Lancet 2003; 362: 1011-1016.
15. De Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, van der Hoeven JG, Pickkers P, Bogaers-Hofman D, van der Meer NJ, Bernards AT, Kuijper EJ, Joore JC, Leverstein-van

Hall MA, Bindels AJ, Jansz AR, Wesselink RM, de Jongh BM, Dennesen PJ, van Asselt GJ, te Velde LF, Frenay IH, Kaasjager K, Bosch FH, van Iterson M, Thijsen SF, Kluge GH, Pauw W, de Vries JW, Kaan JA, et al. Decontamination of the digestive tract and oropharynx in ICU patients. N Engl J Med 2009; 360: 20-31.

16. Ong DS, Klein Klouwenberg P, Spitoni C, Bonten MJ, Cremer OL. Nebulised amphotericin B to eradicate Candida colonisation from the respiratory tract in critically ill patients receiving selective digestive decontamination: a cohort study. Crit Care 2013; 17: R233.
17. Van 't Veen A, Gommers D, Mouton JW, Kluytmans JA, Krijt EJ, Lachmann B. Exogenous pulmonary surfactant as a drug delivering agent: influence of antibiotics on surfactant activity. Br J Pharmacol 1996; 118: 593-598.
18. Ruijgrok EJ, Vulto AG, Van Etten WM. Efficacy of aerosolized amphotericin B desoxycholate and liposomal amphotericin B in the treatment of invasive pulmonary aspergillosis in severely immunocompromized rats. J Antimicrob Chemother 2001; 48: 89-95.
19. Knechtel SA, Klepser ME. Safety of aerolized amphotericin B. Expert Opin Drug Saf 2007; 6: 523-532.
20. Fauvel M, Farrugia C, Tsapis N, Gueuti C, Cabaret O, Bories C, Bretagne S and Barratt G. Aerosolized liposomal amphotericin B: prediction of lung deposition, in vitro uptake and cytotoxicity. Int J Pharm 2012; 436: 106-110.
21. Monforte V, López-Sánchez A, Zurbano F, Usseti P, Sole A, Casals C, Cifrian J, de Pablos A, Bravo C and Roman A. Prophylaxis with nebulized liposomal amphotericin B for Aspergillus infection in lung transplant patients does not cause changes in the lipid content of pulmonary surfactant. J Heart Lung Transplant 2013; 32: 313-319.
22. Grgurich PE, Hudcova J, Lei Y, Sarwar A, Craven DE. Diagnosis of ventilator-associated pneumonia: controversies and working toward a gold standard. Curr Opin Infect Dis 2013; 26: 140-150.
23. Rosbolt MB, Sterling ES, Fahy BG. The utility of the clinical pulmonary infection score. J Intensive Care Med 2009; 24: 26-34.
24. Murray JF, Matthay MA, Luce JM Flick MR. An expanded definition of the adult respiratory distress syndrome. Am Rev Respir Dis 1988; 138: 720-723.
25. Hamil RJ. Amphotericin B formulations: a comparison review of efficacy and toxicity. Drugs 2013; 73: 919-934.

Chapter 7

Echinocandin to fluconazole step-down therapy in critically ill patients with invasive, susceptible *Candida albicans* infections

Patrick J. van der Geest,[1] Bart J.A. Rijnders,[2] Alieke G. Vonk,[2] and A.B. Johan Groeneveld.[1]

[1]Department of Intensive Care medicine, Erasmus MC, Rotterdam, the Netherlands.
[2]Department of Medical Microbiology, Erasmus MC, Rotterdam, the Netherlands.

Mycoses 2016; 59: 179-85.

Abstract

Background

Invasive *Candida* spp. infections are increasingly diagnosed in critically ill patients. For initial treatment, an echinocandin is recommended with a possible step-down to fluconazole when the patients' condition is improving and the isolate appears susceptible, but there are no data to support such policy. We studied the safety and efficacy of step-down therapy in critically ill patients with culture proven deep seated or bloodstream infections by *C. albicans* susceptible to fluconazole.

Methods

All patients admitted into the intensive care unit from January 2010 to December 2014, who had a culture proven invasive *C. albicans* infection and received initial treatment with an echinocandin for at least 4 days were included. Data on patient characteristics, treatment and vital outcomes where assessed.

Results

Of the 56 patients, 32 received step-down fluconazole therapy, at median day 5, whereas the echinocandin was continued in the other 24. No differences were seen in baseline characteristics or risk factors for invasive *C. albicans* infection between the two groups. Response rates were similar and no difference where seen in 28-day or 90-day mortality between the groups.

Conclusion

Step-down therapy to fluconazole may be safe and effective in critically ill patients with invasive infections by *C. albicans,* susceptible to fluconazole, who have clinically improved as early as 4 days after start of treatment with an echinocandin.

Introduction

Invasive fungal infections caused by *Candida* spp. are increasingly common in critically ill patients even when non-neutropenic [1-7]. These infections are associated with a prolonged hospital stay and increased mortality [1,4,8,9]. Early treatment with an appropriate antifungal drug is mandatory since mortality of candidaemia is directly correlated with delayed antifungal therapy [10,11].

Guidelines recommend to start with an echinocandin for treatment of critically ill patients with proven invasive infection caused by *Candida* spp. [12,13]. Step-down therapy to fluconazole is advised for patients who have improved clinically after initial therapy with an echinocandin and in whom fluconazole susceptible *Candida* spp. (e.g. *C. albicans, C. parapsilosis, and C. tropicalis*) have been documented [12,13], although some studies suggest an improved outcome with continued echinocandin treatment [7,14-17]. Three other studies support step-down to (oral) fluconazole therapy after initial treatment of variable duration with an echinocandin in patients with candidaemia or invasive candidiasis caused by susceptible *C. albicans* isolates [18-20]. However, their conclusion is based on expert opinion [18] or on results that were mostly obtained in non-ICU patients [19,20].

We therefore aimed to study the step-down strategy to intravenous and oral fluconazole after initial treatment with an echinocandin for invasive and susceptible *C. albicans* in the critically ill. We hypothesized that such strategy is safe and effective, even if done relatively early after start of treatment. We therefore studied response rates and disease severity-adjusted mortality among patients with and without such step-down treatment.

Patients and methods

Patients

Data were retrospectively collected from our patient data management system, according to a predefined checklist. All patients above 18 years of age diagnosed with deep seated or bloodstream infection by *C. albicans* and who received antifungal treatment starting with an echinocandin (caspofungin, anidulafungin or micafungin) in the Intensive Care Unit (ICU) of the Erasmus Medical Center Rotterdam, were included when admitted between January 2010 and December

2014. The ICU is a tertiary care mixed medical-surgical ICU with 2000 admissions per year. Candidaemia was defined as at least one blood culture drawn from a peripheral vein positive for *C. albicans* and invasive candidiasis was defined as *C. albicans* positive culture obtained from a normally sterile site, such as pleural or peritoneal fluid, in the context of pleural exudate/empyema, and secondary or tertiary peritonitis following a ruptured viscus and surgery, respectively [18]. In addition, the presence of one or more of the following signs and symptoms of infection was also required: fever or hypothermia; hypotension; localized signs and symptoms of inflammation; or radiological findings of invasive candidiasis. There were 124 patients diagnosed with invasive candidiasis from January 2010 until December 2014 and 49 patients had invasive candidiasis with *C. non-albicans* species. All patients were initially treated with an echinocandin. Hence. there were 75 patients with invasive *C. albicans* infection who received initial treatment with an echinocandin, but we selected patients (n=56) who received echinocandin treatment for 4 days or more, since the average time to obtain culture results with susceptibility pattern takes approximately 96 hours. Hereby we eliminated patients with early death during echinocandin treatment, so that the remaining population had a proper chance for step-down therapy to fluconazole. Informed consent was not needed for this retrospective patient data analysis, according to Dutch law, because data had been collected during routine clinical care and were treated anonymously, and the purpose of this study is to evaluate a standard clinical care policy of unknown value.

Clinical protocol

Patients were taken care of by attending intensivists according to national and local guidelines. Selective decontamination of the digestive tract (SDD) is routinely used in our center for patients expected to remain on mechanical ventilation for >48 hours. This involves the administration of the non-absorbable antimicrobial agents tobramycin, amphotericin-B and colistin. Patients also received cefotaxim 1 gram intravenously q.i.d. for a three-day period. As part of this protocol, inventory cultures are taken of the throat, tracheal aspirates, and rectum on admission. To monitor the effect of SDD treatment, surveillance cultures (from throat, tracheal aspirates, and rectum) were routinely done thrice weekly. The SDD cultures were screened for the presence of Gram-negative rods, and yeast that were identified to the species level (see below). At the discre-

tion of the attending intensivist, additional cultures were taken from a possible source of infection, including blood cultures. For fungal detection, all materials obtained from a normally sterile site were cultured onto relevant agar plates for the detection of both bacteria and yeast. Blood culture bottles, specific for the recovery of yeast (BACTEC Mycosis IC/F), that became positive were subcultured onto chocolate agar, Sabouraud agar, and CHROMagar™ to ensure purity or mixed infection and differentiation of yeast, and incubated at 35 °C. As soon as visible growth of a *Candida* colony form a normally sterile site was observed it was identified to the species level using auxacolor (Sanofi Diagnostics Pasteur) or MALDI-TOF. Susceptibility testing of isolates obtained from normally sterile sites was performed using a CLSI broth microdilution method (Sensititre®, Thermoscientific, USA) and results for azoles, amphotericin B and caspofungin, were reported according to revised species-specific CLSI clinical breakpoints. For fluconazole, *C. albicans* was considered susceptible if the minimum inhibitory concentration (MIC) was ≤2 mg/L, and reduced susceptibility was defined as a MIC of ≥4 mg/L [21], after 24-48 hours of growth. The start and step-down to antifungal treatment was at the discretion of the attending intensivist in close collaboration with infectious disease specialists. The first echinocandin we used in the department was caspofungin, followed by anidulafungin and micafungin, exclusively used in 2013 and thereafter. For caspofungin patients received an intravenous daily dose of 50 mg after an initial single loading dose of 70 mg, adjusted for body weight and liver function. Patients receiving intravenous anidulafungin obtained a single loading dose of 200 mg followed by 100 mg daily dose. Micafungin was administered 100 mg intravenous once daily without a loading dose. The step-down from an echinocandin to fluconazole was done when patients were considered to no longer need echinocandin treatment (i.e. clinical improvement, as defined below) and the cultured *Candida* spp. appeared susceptible to fluconazole. Fluconazole was given orally in patients with a normal gastrointestinal transit. Patients receiving intravenous (n=22) or oral fluconazole (n=10) obtained a single loading dose of 800 mg followed by a daily dose of 400 mg. Fluconazole therapy was adjusted for renal function, and patients with a creatinine clearance of 50 ml per minute or less received 200 mg per day. Patients receiving renal replacement therapy obtained a daily dose of 400 mg after 800 mg loading dose. Removal of IV catheters suspected to be the origin of infection was routinely done as well as drainage of suspected pus

collections. The duration of the antifungal therapy was decided in close collaboration with the infectious diseases physician, based on the Dutch invasive fungal infection guidelines which take several factors into account, such as the duration of positive cultures, the certainty of good drainage and clinical improvement, including absence of fever for >24 hours and haemodynamic stability, and neutropenia [12,13,22].

Study protocol and data collection

On admission, baseline demographic data and clinical variables, including age, sex, pre-morbidity, reasons of admission, use of antibiotics including SDD, antifungal treatment, steroids, immunosuppressive medication, immune status (active malignancy or other causes of an immunocompromised state), and risk factors for invasive *Candida* spp. infection (neutropenia, recent surgery, diabetes mellitus, cancer, mechanical ventilation, renal replacement therapy, total parenteral nutrition, transplant and central venous catheters) were recorded. The acute physiology and chronic health evaluation II (APACHE II) and the sequential organ failure assessment (SOFA) score were recorded on admission. To monitor clinical improvement the SOFA score was also calculated at day 1 and 4 after start of an echinocandin, since culture results with susceptibility pattern became available at day 4 and step-down therapy was initiated at median day 5. For each patient a *Candida* score was calculated at the start of echinocandin treatment to help, when >3, establishing the risk for invasive candidiasis vs colonization [23]. The primary site of infection and the obtained culture results from admission until ICU death or discharge were recorded. A global response at the end of treatment was defined as both clinical success (cure – resolution of signs and symptoms of *C. albicans* infection, or improvement – incomplete resolution of signs and symptoms of *C. albicans* infection) and microbiological success (2 negative follow-up cultures for *C. albicans*, or presumed eradication when the follow-up culture was not available and clinical response was defined as cure or improvement at the end of treatment). The duration of *C. albicans* infection was defined as the period between the first positive culture and the first negative culture or ICU death or discharge. Duration of treatment was defined as the period between initiation and stop of antifungal therapy or ICU death or discharge. Patients were followed until day 28 and 90 after start of antifungal therapy and length of ICU stay and vital outcomes were recorded.

Statistical analysis

There was no formal power calculation for this retrospective modified intention-to-treat analysis. Continuous variables were expressed as median and interquartile range since the assumption of normality was often violated (Kolmogorov-Smirnov test, $P<0.05$). The Mann-Whitney U test was used for continuous data and the Fisher exact test for categorical data. Kaplan-Meier curves were constructed and log rank testing was performed to evaluate group differences in survival. Multivariable Cox regression analysis was done to evaluate determinants of mortality including step-down therapy. All tests were 2 sided, and $P<0.05$ was considered statistically significant. Exact *P* values >0.001 are shown.

Results

Fifty-six patients with invasive *C. albicans* infections received an echinocandin for 4 or more days. Of those patients, 32 received step-down fluconazole therapy (Group A) whereas the echinocandin was continued in the other 24 (Group B). No differences were seen in characteristics on admission and at the start of the echinocandin treatment between the groups (Table 1).

Infection and treatment characteristics

Of the 56 patients, 19 patients had candidaemia, 32 patients had abdominal and 5 patients had pleural infection (Table 2). There were no patients with meningitis, endophthalmitis or osteomyelitis. Both groups had a *Candida* score 3 on average. Fluconazole was started at median day 5 after start of echinocandin treatment in Group A, so that the echinocandin was administered for 5 days or more in 22 of 32 patients. The *C. albicans* isolates susceptible to fluconazole had a median MIC of 0.25 mg/L in Group A and 0.33 mg/L in Group B. In Group B 2 isolates where fluconazole identified as less susceptible with an intermediary clinical breakpoint of 4 mg/L. There were no echinocandin-resistant strains. Durations of infection and treatment, and successful response rates where comparable between the groups. The difference in global response rate between Group A and B was -2% (95% confidence interval [95% CI] -25 to 22%). The difference in clinical response rates was -8% (95% CI -24 to 9%). The difference in microbial response rates between groups was 2% (95% CI -18 to 24%).

The length of ICU stay was comparable for both groups. No differences were observed in 28-day or 90-day mortality (Table 1 and Fig. 1). The difference in 28-day mortality between Group A and B was -16% (95% CI -39 to 10%).

Table 1. Demographic and clinical characteristics at baseline and start of echinocandin for invasive *Candida albicans* infection.

	Group A	**Group B**	**P**
	(n=32)	**(n=24)**	
On admission			
Age (years)	59 (20)	65 (21)	0.17
Gender (male)*	20 (63)	15 (63)	0.99
APACHE II score	25 (14)	28 (13)	0.24
SOFA score	10 (6)	10 (6)	0.87
Reasons of ICU admission*			0.46
Suspected sepsis	15 (47)	13 (54)	
Respiratory failure	2 (6)	2 (8)	
Renal failure	2 (6)	2 (8)	
Liver failure	2 (6)	1 (4)	
CPR	0 (0)	0 (0)	
Shock	1 (3)	3 (13)	
Postoperative	10 (31)	3 (13)	
At start of echinocandin			
Risk factors for invasive *Candida* spp. Infection*			
Neutropenia	1 (3)	2 (8)	0.40
Broad-spectrum antibiotics	12 (38)	10 (42)	0.75
Immunosuppression	0 (0)	1 (3)	0.39
Steroids	12 (38)	9 (38)	0.99
TPN	6 (19)	4 (17)	0.84
Recent surgery	15 (47)	11 (46)	0.94
DM II	5 (16)	5 (21)	0.62
Malignancy	8 (25)	5 (21)	0.72
Transplant	4 (13)	3 (13)	0.99
Ventilation	31 (97)	22 (92)	0.40
CVVH	10 (31)	11 (45)	0.09
Central venous catheter	30 (94)	22 (92)	0.77
Duration between admission and start of echinocandin (days)	4 (6)	2 (4)	0.06
Duration between start of echinocandin and step-down (days)	5 (3)	n.a.	

*Numbers (percentage) or median (interquartile range), where appropriate. Group A: patient who stepped-down to fluconazole. Group B: patients only treated with an echinocandin. Abbreviations: APACHE II = Acute Physiology and Chronic Health Evaluation II; CPR = cardiopulmonary resuscitation; CVVH = continuous veno-venous haemofiltration; DM II = diabetes mellitus type II; ICU = intensive care unit; SOFA = sequential organ failure assessment score; TPN = total parenteral nutrition; n.a.= not applicable.

Multivariable analysis

In multivariable Cox regression analysis, the SOFA score at day 4 was the best parameter (hazard ratio 1.18 95% confidence interval 1.06-1.31, P<0.002) to predict 90-day mortality, whereas age, APACHE II, SOFA score on admission, *Candida* score and step-down therapy did not contribute (P>0.05).

Table 2. Infection and treatment characteristics.

	Group A	**Group B**	**P**
	(n=32)	**(n=24)**	
Temperature at start, °C	37.7 (0.9)	37.7 (1.0)	0.60
Temperature day 4, °C	36.7 (0.9)	36.8 (0.8)	0.99
SOFA at start	12 (9)	13 (6)	0.11
SOFA day 4	7 (9)	9 (9)	0.12
Candida score at start	3 (3)	4 (1)	0.15
Source of isolates*			
Blood	13 (41)	6 (25)	0.43
Intra-abdominal fluid	16 (50)	16 (67)	
Pleural fluid	3 (9)	2 (8)	
Antifungal therapy*			
Anidulafungin	18 (56)	14 (58)	0.54
Caspofungin	4 (13)	3 (13)	0.65
Micafungin	10 (31)	7 (29)	0.55
MIC fluconazole, mg/L	0.25 (0.25)	0.33 (0.25)	0.87
Global response*	22 (69)	17 (71)	1.0
Clinical response*	28 (87)	23 (96)	0.68
Microbial response*	26 (81)	19 (79)	0.85
Gaining negative cultures*	22 (69)	15 (63)	0.78
Duration of infection (days)	4 (8)	3 (8)	0.97
Duration of treatment (days)	17 (12)	14 (13)	0.08
Length of ICU stay (days)	22 (26)	21 (16)	0.36
Mortality day 28 after start*	11 (34)	12 (50)	0.16
Mortality day 90 after start*	16 (50)	15 (63)	0.36

*Numbers (percentage) or median (interquartile range), where appropriate. Group A: patients who stepped-down to fluconazole. Group B: patients only treated with an echinocandin. Abbreviations: ICU = intensive care unit; MIC = minimal inhibitory concentration; SOFA = sequential organ failure assessment score.

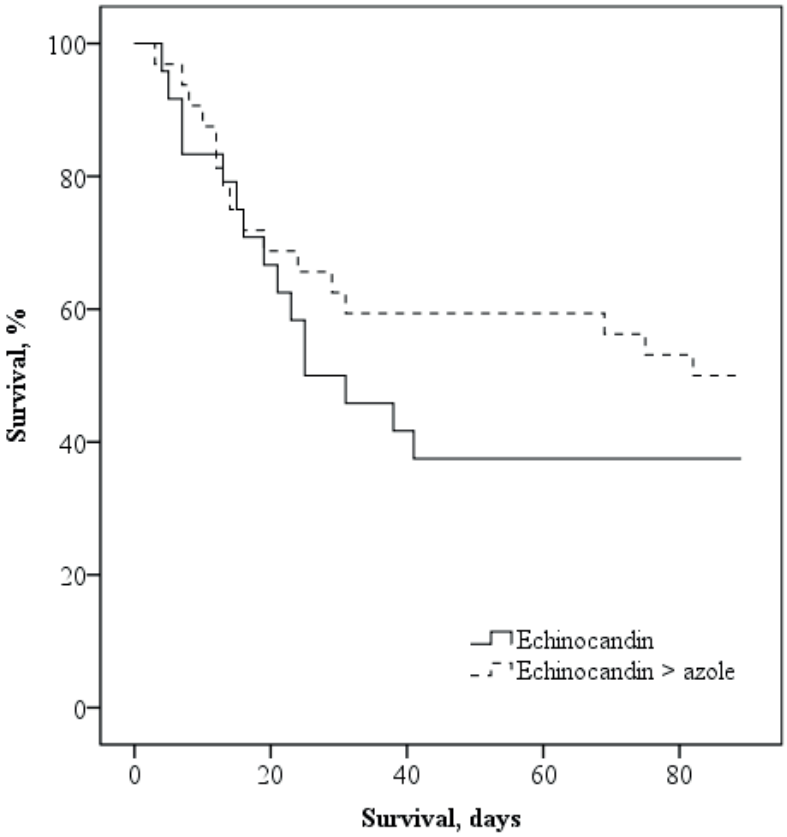


Figure 1. Kaplan–Meier survival curve up to day 90 after initiation of antifungal therapy. Patient who stepped-down (>) to fluconazole(Group A) vs. those only treated with an echinocandin (Group B); P = 0.35 (log-rank test).

Discussion

This study suggests that step-down therapy to fluconazole can be safely and effectively applied in critically ill patients with invasive, fluconazole-susceptible *C. albicans* infections. No difference was seen in 28-day or 90-day mortality, with step-down to fluconazole versus continued echinocandin treatment after the first 4 days of echinocandin treatment.

The type and duration of treatment of invasive *Candida* spp. infections depends on the result of cultures and sensitivity testing, and the extent of organ involvement and patients' clinical condition [12,13,18]. Three of 56 (5%) *C. albicans* isolates in this study where non-susceptible to fluconazole, in line with the literature [4,24], whereas MIC's of fluconazole to *C. albicans* were similar to

those in previous studies [14,16]. In patients with candidaemia or deep seated infection, continued therapy for 14 days after the first negative blood culture is recommended [12,13,18], which is in line with the observed length of treatment of 14-17 days in this study. Overall response rates in this study are comparable to those found in other studies [14-16,19,20]. Some experts and guidelines suggest to step-down to fluconazole therapy only after 5-10 days of echinocandin treatment [12-14,18,20]. Our results indeed suggest that this can already be done by day 5.

The results of this study are comparable to previous studies on the safety and efficacy of step-down therapy in mainly non-ICU patients [19,20]. In the largest (n=282) published study on step-down therapy that we are aware of [20], the APACHE II score in the step-down population was 12 and therefore lower than 25 in ours. Also, this study was mostly limited to non-ICU patients as only 23 patients with early step-down treatment, a population comparable to ours, were in the ICU for >4 days. Finally, the study [20] also included patients with *C. non-albicans* spp. and step-down therapy to oral voriconazole. It separately studied early step-down therapy to (only oral) fluconazole in susceptible *C. albicans* infections (n=41), but did not evaluate its mortality as we did. Therefore, our study adds relevant data on step-down therapy in critically ill patients and suggests that this strategy is safe as soon as susceptibility is documented and the patient has clinically improved during echinocandin therapy. Our retrospective data are in line with the most recently published post hoc data from the AmarCAND2 study in France, suggesting that de-escalation of systemic antifungal therapy for suspected or documented invasive candidiasis is safe and not associated with increased mortality [25]. The overall 28-day mortality in our study was 41% and comparable to the mortality observed in other studies [3,4,7,16].

The step-down therapy we studied has several advantages. Depending on the local resale price of the echinocandins, there may be substantial cost savings as a result of the step-down therapy [19,26,27]. Furthermore, fluconazole has an excellent bioavailability and can be given orally in patients with a normal gastrointestinal transit [12,19,20]. Finally, as expected, acquired resistance during prolonged treatment of ICU patients with echinocandins has been observed [24]. Studies suggest that already after seven days of echinocandin exposure resistance associated FKS mutations can be observed [28]. Applying a step-down strategy to fluconazole decreases the echinocandin exposure time

and may partially prevent the occurrence of these mutations and the risk for breakthrough infections with echinocandin resistant *Candida* spp. [29].

Our study has several limitations and results should be interpreted cautiously. The number of patients included in this study was relatively small, so that results should be regarded as preliminary. This study enrolled 75 patients with invasive candidiasis by *C. albicans* in a four-year period in a tertiary care ICU with 2,000 admissions per year. Hence, the reported incidence of invasive candidiasis was 10 per 1,000 ICU admissions and comparable to the incidence of 5 to 10 cases per 1,000 ICU admissions in a large review [5]. *C. albicans* is the most cultured *Candida* spp. responsible for invasive fungal infection, however a shift to azole non-susceptible isolates and the emergence of resistant *C. non-albicans* spp. has been noted [2-5,7,8,15,24,30]. Therefore, a step-down therapy may become less applicable in centers with a high incidence of resistant *C. (non-)albicans* spp. infections. Obviously, there is patient selection in this study of step-down therapy, but SOFA day 4 was a major determinant of outcome, irrespective of *Candida* score or step-down therapy, thereby suggesting that the latter was not only done in patients with relatively high overall survival chances. Otherwise, the objective clinical course did not differ between groups until day 4, when clinicians decided on step-down therapy in many (Group A), but not all (Group B), patients, on the basis of clinical improvement with absence of fever >24 h and hemodynamic stability. We could not retrospectively further capture the arguments on which this decision was taken. It is unlikely that a sufficiently powered randomized study to confirm our retrospective findings will ever be performed.

In conclusion, step-down therapy to fluconazole may be a safe and effective approach in critically ill patients with candidaemia or invasive candidiasis caused by fluconazole susceptible *C. albicans*, who have clinically improved as early as 4 days after start of treatment with an echinocandin.

References

1. Kett DH, Azoulay E, Echeverria PM, Vincent JL. Candida bloodstream infections in intensive care units: analysis of the extended prevalence of infection in intensive care unit study. Crit Care Med 2001; 39: 665-670.
2. Chow JK, Golan Y, Ruthazer R, Karchmer AW, Carmeli Y, Lichtenberg D, et al. Factors associated with candidemia caused by non-albicans *Candida* species versus *Candida albicans* in the intensive care unit. Clin Infect Dis 2008; 46: 1206-1213.
3. Guery BP, Arendrup MC, Auzinger G, Azoulay E, Borges Sa M, Johnson EM, et al. Management of invasive candidiasis and candidemia in adult non-neutropenic intensive care unit patients: Part I. Epidemiology and diagnosis. Intensive Care Med 2009; 35: 55-62.
4. Leroy O, Gangneux JP, Montravers P, Mira JP, Gouin F, Sollet JP, et al. AmarCand study group. Epidemiology, management, and risk factors for death of invasive Candida infections in critical care: a multicenter, prospective, observational study in France (2005-2006). Crit Care Med 2009; 37: 1612-8.
5. Eggimann P, Bille J and Marchetti O. Diagnosis of invasive candidiasis in the ICU. Ann Intensive Care 2011; 1: 37.
6. Glöckner A. Treatment and prophylaxis of invasive candidiasis with anidulafungin, caspofungin and micafungin – review of the literature. Eur J Med Res 2011; 16: 167-179.
7. Colombo AL, Guimaraes T, Sukienik T, Pasqualotto AC, Andreotti R, Queiroz-Telles F, et al. Prognostic factors and historical trends in the epidemiology of candidemia in critically ill patients: an analysis of five multicenter studies sequentially conducted over a 9-year period. Intensive Care med 2014; 40: 1489-1498.
8. Dimopoulos G, Ntziora F, Rachiotis G, Armaganidis A, Falagas ME. Candida albicans versus non-candida albicans intensive care unit-acquired bloodstream infection: differences in risk factors and outcome. Anesth Analg 2008; 106: 523-529.
9. Prowle JR, Echeverri JE, Ligabo EV, Sherry N, Taori GC, Crozier TM, et al. Acquired bloodstream infection in the intensive care unit: incidence and attributable mortality. Crit Care 2011; 15: R100.
10. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. Antimicrob Agents Chemother 2005; 49: 3640-3645.
11. Garey KW, Rege M, Pai MP, Mingo DE, Suda KJ, Turpin RS, et al. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi institutional study. Clin Infect Dis 2006; 43: 25-31.
12. Pappas PG, Kauffman CA, Andes D, Benjamin DK, Calandra TF, Edwards JE, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 2009; 48: 503-535.
13. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortolary O, et al. ESCMID guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. Clin Microbiol Inf 2012; 18: 19-37.
14. Reboli AC, Rotstein C, Pappas PG, Chapman SW, Kett DH, Kumar D, et al. Anidulafungin versus fluconazole for invasive candidiasis. N Engl J Med 2007; 356: 2472-2482.
15. Kett DH, Shorr AF, Reboli AC, Reisman AL, Biswas P, Schlamm HT. Anidulafungin compared with fluconazole in severely ill patients with candidemia and other forms of invasive candidiasis: support for the 2009 IDSA treatment guidelines for candidiasis. Crit Care 2009; 15: R253.
16. Reboli AC, Shorr AF, Rotstein C, Pappas PG, Kett DH, Schlamma HT, et al. Anidulafungin compared with fluconazole for treatment of candidemia and other forms of invasive candidiasis caused by *Can-*

dida albicans: a multivariate analysis of factors associated with improved outcome. BMC Inf Dis 2011; 11: 261.

17. Wade RL, Chaudhari P, Campbell RS, Hays HD, Nathanson BH, Yi J, et al. Treatment of candidemia with echinocandins: data on hospital resource use from a real world setting. J Med Econ 2012; 15: 1130-8.
18. Bassetti M, Marchetti M, Chakrabarti A, Colizza S, Garnacho-Montero J, Kett DH, et al. A research agenda on the management of intra-abdominal candidiasis: results from a consensus of multinational experts. Intensive Care Med 2013; 39: 2092-2106.
19. Bal AM, Shankland GS, Scott G, Imtiaz T, Macaulay R, McGill M. Antifungal step-down therapy based on hospital intravenous to oral switch policy and susceptibility testing in adult patients with candidaemia: a single centre experience. Int J Clin Pract 2014; 68: 20-27.
20. Vazquez J, Reboli AC, Pappas PG, Patterson TF, Reinhardt J, Chin-Hong P, et al. Evaluation of an early step-down strategy from intravenous anidulafungin to oral azole therapy for the treatment of candidemia and other forms of invasive candidiasis: results from an open-label trial. BMC Inf Dis 2014; 14: 97.
21. Clinical and Laboratory Standards Institute (CLSI), 2012 Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts: 4th informational supplement. CLSI, Wayne, PA (2012)
22. Oude Lashof AML, Janssen JJWM, Meis JFGM, Warris A, Wout JW van 't, Natsch SS, et al. SWAB-richtlijn voor de behandeling van invasieve schimmelinfecties 2013. NVMM 2013; 18: 13-17.
23. León C, Ruiz-Santana S, Saavedra P, Galván B. Blanco A, Castro C, et al. Usefulness of the '*Candida* score' for discriminating between *Candida* colonization and invasive candidiasis in non-neutropenic critically ill patients: A prospective multicenter study. Crit Care Med 2009; 37: 1624-1633.
24. Maubon D, Garnaud C, Calandra T, Sanglard D, Cornet M. Resistance of Candida spp. To antifungal drugs in the ICU: where are we now? Intensive Care Med 2014; 40: 1241-1255
25. Bally S, Leroy O, Montravers P, Constantin J-M, Dupont H, Guillemot D, et al. Antifungal de-escalation was not associated with adverse outcome in critically ill patients treated for invasive candidiasis: post hoc analyses of the AmarCAND2 study data. Intensive Care Med 2015;41:1931-1940.
26. Wilke MH. Treatment and prophylaxis of invasive candidiasis with anidulafungin, caspofungin and micafungin and its impact on use and costs – review of the literature. Eur J Med Res 2011; 16: 180-186.
27. Masterton RG, Casamayor M, Musingarimi P, van Engen A, Zinck R, Odufowora-Sita O, et al. De-escalation from micafungin is a cost-effective alternative to traditional escalation from fluconazole in the treatment of patients with systemic Candida infections. J Med Econ 2013; 16: 1344-56.
28. Alexander BD, Johnson MD, Pfeiffer CD, Jimenez-Ortigosa C, Catania J, Booker R, et al. Increasing echinocandin resistance in *Candida glabrata:* clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. Clin Infect Dis 2013; 56: 1724-1732.
29. Pfeiffer CD, Garcia-Effron G, Zaas AK, Perfect JR, Perlin DS, Alexander BD. Breakthrough invasive candidiasis in patients on micafungin. J Clin Microbiol 2010; 48: 2373-2380.
30. Bassetti M, Ansaldi F, Nicolini L, Malfatto E, Molinari MP, Mussap M, et al. Incidence of candidemia and relationship with fluconazole use in an intensive care unit. J Antimicrob Chemother 2009; 64: 625-629.

Chapter 8

Micafungin versus anidulafungin in critically ill patients with invasive candidiasis: a retrospective study

Patrick J. van der Geest,[1] Nicole G. M. Hunfeld,[2] Sophie E. Ladage,[1] and A.B. Johan Groeneveld.[1]

[1]Department of Intensive Care medicine, Erasmus MC, Rotterdam, the Netherlands.
[2]Department of Pharmacy, Erasmus MC, Rotterdam, the Netherlands.

BMC Infectious Diseases 2016; 16: 490.

Summary

Background

In critically ill patients the incidence of invasive fungal infections caused by *Candida spp.* has increased remarkably. Echinocandins are recommended as initial treatment for invasive fungal infections. The safety and efficacy of micafungin compared to caspofungin is similar, but no comparison is made between anidulafungin and micafungin concerning safety and efficacy. We therefore performed a retrospective study to assess these aspects in critically ill patients with invasive candidiasis.

Methods

All patients in the intensive care unit (ICU) with invasive candidiasis, who were only treated with anidulafungin or micafungin, between January 2012 and December 2014 were retrospectively included. Baseline demographic characteristics, infection characteristics and patient courses were assessed.

Results

A total of 63 patients received either anidulafungin (n=30) or micafungin (n=33) at the discretion of the attending intensivist. Baseline characteristics were comparable between the two groups, suggesting similar risk for developing invasive candidiasis. Patients with invasive candidiasis and liver failure were more often treated with anidulafungin than micafungin. Response rates were similar for both groups. No difference was observed in 28-day mortality, but 90-day mortality was higher in patients on anidulafungin. Multivariable cox regression analysis showed that age and serum bilirubin were the best parameters for the prediction of 90-day mortality, whereas APACHE II, Candida score and antifungal therapy did not contribute ($P>0.05$). None of the patients developed impaired liver function related to antifungal use and no differences were seen in prothrombin time, serum transaminases and bilirubin levels between the groups, after exclusion of patients with liver injury or failure.

Conclusion

Micafungin can be safely and effectively used in critically ill patients with invasive candidiasis. The observed increased 90-day mortality with anidulafungin can be explained by intensivists unnecessarily avoiding micafungin in patients with liver injury and failure.

Background

The incidence of invasive fungal infections caused by *Candida spp*. is increasing in critically ill patients [1,2]. The latter is associated with prolonged duration of hospitalization and higher mortality rates [3-5]. The mortality of invasive candidiasis is directly correlated with a delay in starting antifungal therapy and therefore early treatment with an appropriate antifungal drug is mandatory [6,7].

A relatively novel class of antifungal agents are echinocandins which have activity against a broad spectrum of *Candida spp.,* including *C. glabrata* and *C. krusei*, against which fluconazole has less activity [8]. Echinocandins are recommended as initial treatment for invasive candidiasis in patients with moderate to severe illness, keeping fluconazole reserved for less critically ill patients[9]. The recommendation are based on prospective randomized clinical trials which demonstrated that all three echinocandins are at least as effective as fluconazole for the treatment of invasive candidiasis [10-15]. The antifungal activity, pharmacokinetics and toxicity profile of each echinocandin is slightly different, but the relevance of this finding remains unclear [16]. Both caspofungin and micafungin undergo hepatic metabolism, in contrast to anidulafungin, which undergoes spontaneous degradation [17]. Concerns about possible hepatotoxicity of micafungin have been raised which may affect its use in daily practice [2]. Two randomized controlled trials [16,18] and one retrospective observational trial [19], compared the safety and efficacy of micafungin versus caspofungin in the treatment of invasive candidiasis, showing similar results in the safety and efficacy of micafungin compared with caspofungin. One systematic review including 8,000 patients [20] and one observational cohort study with 8,696 patients [21], evaluated the safety of micafungin versus other echinocandins, showing no increased risk of hepatic injury by micafungin. Both studies only evaluated the safety concerning hepatotoxicity of antifungal medication in mostly non-ICU patients, but did not evaluate mortality or efficacy.

As far as we are aware there are no studies comparing the efficacy and safety of micafungin versus anidulafungin in critically ill patients with invasive candidiasis. We performed a retrospective study to compare the safety and efficacy of micafungin versus anidulafungin in critically ill patients with invasive candidiasis.

Methods

Patients

We retrospectively gathered data from the patients' medical records using a predefined checklist. Between January 1, 2012, and January 1, 2015, all patients over the age of 18 with invasive candidiasis and who only received anidulafungin or micafungin as systemic antifungal treatment in the Intensive Care Unit (ICU) of the Erasmus University Medical Center Rotterdam, were considered for participation. Candidemia was defined as at least one positive blood culture for *Candida spp*. drawn from a peripheral vein. Invasive candidiasis was defined as a positive culture with *Candida spp*. obtained from a normally sterile site, such as pleural or peritoneal fluid, in the context of pleural exsudate/empyema, and secondary or tertiary peritonitis following a ruptured viscus and surgery, respectively [22]. In addition, patients needed to have one or more of the following signs and symptoms of infection: fever or hypothermia; hypotension; localized signs and symptoms of inflammation; or radiological findings of invasive candidiasis. Between January 2012 and December 2014 there were 124 patients diagnosed with invasive candidiasis of whom 20 received caspofungin, 38 stepped-down to fluconazole and 3 received both antifungals. In total there were 63 patients with invasive *Candida spp*. infection who only received anidulafungin or micafungin. The Dutch law states that informed consent is not required in case of retrospective analysis in which data collected during routine clinical care were used and anonymously analyzed.

Clinical protocol

Patients were taken care of by attending intensivists according to national and local guidelines. In our center selective decontamination of the digestive tract (SDD) is routinely used for patients with an expected duration of mechanical ventilation for more than 48 hours. This involves administration of an oral paste and of a suspension via the nasogastric tube, containing the non-absorbable antibiotics tobramycin, amphotericin-B and colistin. Patients also received cefotaxime intravenously at 4 times 1 gram a day for a three-day period. Inventory cultures are taken of the throat, tracheal aspirates, and rectum as part of this protocol on admission. To monitor the effect of SDD treatment, surveillance cultures (from throat, tracheal aspirates, and rectum) were routinely performed

three times per week. All SDD cultures were screened for the presence of Gram-negative rods, and yeast that were identified to the species level (see below). In case a patient is suspected for having an infection, additional cultures (besides the routinely SDD cultures) can be taken from the possible source of infection, which includes the use of blood cultures. All materials obtained from a normally sterile site were cultured onto relevant agar plates for the detection of both bacteria and yeast. Blood culture bottles, specific for the recovery of yeast (BACTEC Mycosis IC/F), that became positive were subcultured onto chocolate agar, Sabouraud agar, and CHROMagar™ to ensure purity or mixed infection and differentiation of yeast, and incubated at 35 °C. Auxacolor (Sanofi Diagnostics Pasteur) or MALDI-TOF was used to identify the species level of a *Candida* colony as soon as visible growth from a normally sterile site was observed. Susceptibility testing of isolates obtained from normally sterile sites was performed using a CLSI broth microdilution method (Sensititre® , Thermoscientific, USA) and results for azoles, amphotericin B and caspofungin, were reported according to revised species-specific CLSI clinical breakpoints. For fluconazole, *C. albicans* was considered susceptible if the minimum inhibitory concentration (MIC) was ≤2 mg/L, and reduced susceptibility was defined as a MIC of ≥4 mg/L [23], after 24-48 hours of growth. The decision to start an echinocandin was taken by the attending intensivist in collaboration with infectious disease specialist. The initiation of antifungal therapy was based on clinical signs (i.e. fever, hypothermia, hypotension, leukocytosis or leukopenia), risk factors for invasive candidiasis, culture results, radiological findings of invasive candidiasis, and according to published criteria [9]. Caspofungin was introduced in 2001, followed by anidulafungin and micafungin respectively. In case of severe liver injury and liver failure, which was defined as the presence of clinical signs and symptoms of an abnormal liver function (increased liver enzymes, hyperbilirubinemia, coagulopathy and encephalopathy), intensivists prefer anidulafungin over micafungin. In all other instances, the choice between anidulafungin or micafungin was at the discretion of the attending intensivist. For anidulafungin patients received an intravenous daily dose of 100 mg after an initial single loading dose of 200 mg. Micafungin was administered at a dose of 100 mg intravenous once daily without a loading dose. No dose adjustment was needed for body weight or impaired renal or hepatic function. Drainage of suspected pus collections as well as removal of IV catheters suspected to be the

origin of infection was routinely done . The duration of the antifungal therapy was decided in close collaboration with the infectious diseases physician, based on Dutch invasive fungal infection guidelines which take several factors into account, such as duration of positive cultures, the certainty of good drainage and clinical improvement (absence of fever for >24 hours, haemodynamic stability, and neutropenia) [9,24,25].

Study protocol and data collection

Demographic data and clinical date were recorded on admission, including severity of illness scores, risk factors for invasive *Candida spp*. infection (neutropenia, recent surgery, diabetes mellitus, cancer, mechanical ventilation, renal replacement therapy, total parenteral nutrition, transplant and central venous catheters), the duration of ICU stay, and mortality at day 28 and 90 after start of antifungal therapy. Patients were checked for abnormal liver function during antifungal therapy, serum aspartate transaminase (AST) and serum alanine transferase (ALT) were recorded at start and stop of echinocandin treatment. Serum bilirubin, prothrombin time (PT), AST, and ALT peak values were recorded daily during echinocandin treatment. At the start of echinocandin treatment a *Candida* score was calculated for each patient to help, when >3, establishing the risk for invasive candidiasis vs colonization [26]. The primary site of infection and the obtained culture results were recorded. A global response at the end of treatment was defined as both clinical success (cure – resolution of signs and symptoms of *Candida spp*. infection, or improvement – incomplete resolution of signs and symptoms of *Candida spp*. infection) and microbiological success (2 negative follow-up cultures for *Candida spp*., or presumed eradication when the follow-up culture was not available and clinical response was defined as cure or improvement at the end of treatment). The duration of *Candida spp*. infection was defined as the period between the first positive culture and the first negative culture or ICU death or discharge. Duration of treatment was defined as the period between initiation and stop of antifungal therapy or ICU death or discharge.

Statistical analysis

Continuous variables were presented with median and interquartile range because of the not normal distribution (Kolmogorov-Smirnov test, $P<0.05$). Continuous data were examined with The Mann-Whitney U test and categorical

data with the Fisher exact test. The Kaplan-Meier estimation of survival curves (compared by log rank tests) and multivariable cox regression were used for survival analysis. All reported *P* values are two-tailored. Statistical significance was set at P<0.05.

Results

Sixty-three patients with invasive candidiasis received either anidulafungin (n=30) or micafungin (n=33). Patients using anidulafungin were more often on renal replacement therapy (Table 1).

Infection and treatment characteristics

Of the 63 patients, 22 patients had candidemia, 37 patients had abdominal and 4 patients had pleural infection (Table 2). The average *Candida* score was 3 for both groups. Anidulafungin was initiated at median day 3 and micafungin was initiated at median day 2 after ICU admission. There were no echinocandin-resistant strains. Four *C. albicans* isolates were considered less sensible to fluconazole with an average clinical breakpoint of 100 mg/L. There was no difference in the duration of treatment and infection, and response rates, even when corrected for liver failure. Both groups had a similar duration of ICU stay. No difference was observed in 28-day mortality, but 90-day mortality was higher in patients on anidulafungin (Table 2 and Fig 1). Multivariable Cox regression analysis showed that age (hazard ratio 1.13, 95% confidence interval 1.02-1.23, P=0.02) and serum bilirubin (hazard ratio 1.06, 95% confidence interval 1.01-1.12, P=0.03) were the best parameters for the prediction of 90-day mortality, whereas APACHE II, *Candida* score and antifungal therapy did not contribute (P>0.05).

Liver enzymes and function

Patients with invasive candidiasis and liver failure were more often treated with anidulafungin than micafungin. None of the patients in this study developed liver failure or elevated liver enzymes requiring cessation of treatment related to the use of an echinocandin. PT, AST, ALT and bilirubin levels tended to be higher in patients treated with anidulafungin. When excluding liver failure, no differences were seen in PT, AST, ALT and bilirubin levels between the groups (Table 3).

Table 1. Baseline demographic and clinical characteristics.

	Anidulafungin (n=30)	Micafungin (n=33)	P
On admission			
Age (years)	59 (20)	62 (20)	0.36
Gender (male)*	19 (63)	26 (79)	0.18
APACHE II score	26 (10)	23 (8)	0.13
SOFA score	10 (9)	9 (3)	0.63
Reasons of ICU admission*			0.14
Suspected sepsis	9 (30)	11 (33)	
Respiratory failure	2 (7)	6 (19)	
Renal failure	1 (3)	0 (0)	
Liver failure	4 (13)	0 (0)	
CPR	2 (7)	2 (6)	
Shock	5 (17)	3 (10)	
Postoperative	7 (23)	11 (34)	
At start of echinocandin			
Risk factors for invasive candidiasis*			
Neutropenia	3 (10)	2 (6)	0.57
Broad spectrum antibiotics	18 (60)	15 (45)	0.25
Immunosuppression	4 (13)	3 (9)	0.60
Steroids	16 (53)	12 (36)	0.18
TPN	4 (13)	11 (33)	0.07
Recent Surgery	8 (27)	15 (45)	0.13
DM II	11 (37)	7 (21)	0.18
Malignancy	5 (17)	11 (33)	0.13
Transplant	5 (17)	4 (12)	0.61
CVVH	19 (63)	7 (21)	0.001
CVC	29 (97)	29 (88)	0.20
Mechanical ventilation	27 (90)	32 (97)	0.26
Duration between admission and start echinocandin (days)	3 (4)	2 (2)	0.17

Numbers (percentage)* or median (interquartile range), where appropriate. List of abbreviations: APACHE II = Acute Physiology and Chronic Health Evaluation II; CPR = cardiac pulmonary resuscitation; CVVH = continuous venovenous haemofiltration; CVC = central venous catheter; DM II = diabetes mellitus type II; ICU = intensive care unit; TPN = total parenteral nutrition

Costs

Table 4 describes the involved costs for both treatment strategies. The total treatment costs per patient seemed to be higher for micafungin, because of the longer treatment period. However, the treatment costs per patient per day were lower for micafungin.

Table 2. Infection and treatment characteristics

	Anidulafungin (n=30)	Micafungin (n=33)	P
Candida score at start	3 (2)	3 (3)	0.71
Source of isolates*			0.25
Blood	10 (33)	12 (36)	
Intra-abdominal fluid	17 (57)	20 (63)	
Pleural fluid	3 (10)	1 (3)	
*Candida spp.**			0.46
Candida albicans	8 (27)	3 (9)	
Candida dublienis	0 (0)	2 (6)	
Candida glabrata	17 (57)	21 (66)	
Candida krusei	3 (10)	3 (10)	
Candida parapsilosis	1 (3)	2 (6)	
Candida tropicalis	1 (3)	2 (6)	
Global response*	21 (67)	23 (70)	0.80
Clinical response*	24 (80)	28 (85)	0.62
Microbial response*	21 (70)	24 (73)	0.81
Gaining negative cultures*	18 (60)	20 (60)	0.96
Duration of infection (days)	3 (5)	3 (5)	0.80
Duration of treatment (days)	12 (8)	14 (9)	0.40
Length of ICU stay (days)	13 (15)	14 (21)	0.64
Mortality day 28 after start*	20 (67)	18 (55)	0.33
Mortality day 90 after start*	26 (87)	21 (64)	0.04

Numbers (percentage)* or median (interquartile range), where appropriate. Abbreviations: ICU = intensive care unit.

Discussion

This study suggests that in critically ill patients with candidemia or invasive candidiasis, the safety and efficacy of the treatment with micafungin was similar to that of anidulafungin. No differences were seen in response rates, liver function and enzymes, and mortality. Treatment with micafungin seems to be less expensive then that with anidulafungin.

Echinocandins inhibit synthesis of the β-(1-3)-D-glucan compound of the fungal cell wall and are considered as safe drugs [2,17]. The overall efficacy between the three echinocandins is comparable, showing only little difference [27]. Nevertheless, the EMA still recommend to only use micafungin in case other antifungals are not appropriate, as rat experiments suggested a poten-

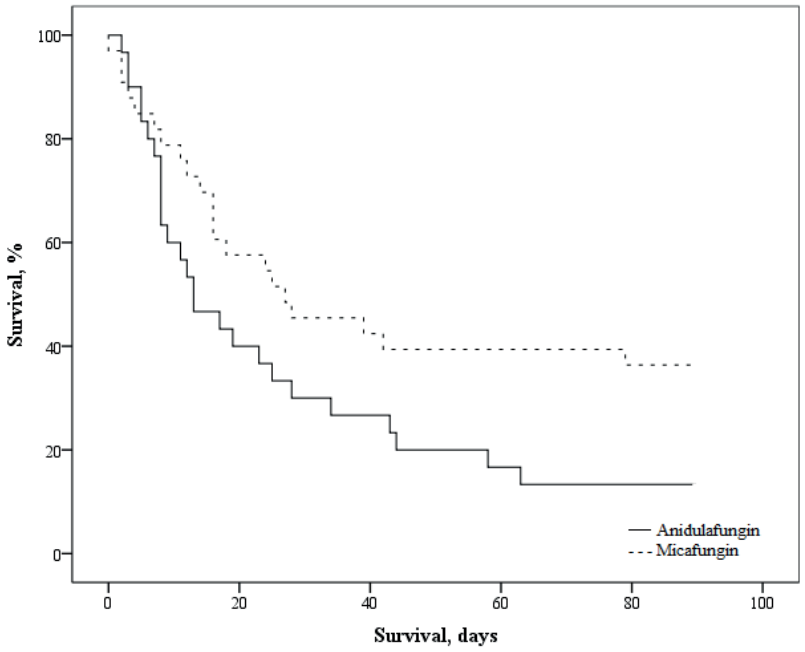


Figure 1. Kaplan-Meier survival curve up to day 90 after initiation of an echinocandin, P=0.04 (log rank test).

tial risk for the development of liver tumors [27]. However, these results were obtained from studies using high dosages for prolonged time in male animals, similar effects were not reported by other studies on humans or animals [17]. Both caspofungin and micafungin undergo hepatic metabolization, in contrast to anidulafungin, which undergoes spontaneous degradation [17]. Transient elevation of liver enzymes occurs in 2 to 15% of patients treated with an echinocandin [28]. In this study we found an elevation in serum AST of 38% and 35%, and an elevation in serum ALT of 33% and 29%, in patients treated with anidulafungin or micafungin, respectively. The observed incidence of elevated liver enzymes in this study is much higher, but equal between both groups of echinocandins, but we only looked at elevated liver enzymes in general and not specifically caused by the echinocandins. Abnormal liver function tests can be found in up to 61% of critically ill patients, as caused by sepsis, drugs or ischemia [29]. Our results suggest that micafungin is as safe as anidulafungin concerning hepatotoxicity. The results are in line with two previous studies, which both concluded that anidulafungin and micafungin had a low risk of elevated liver enzyme levels not requiring the cessation of treatment [20,21]. Both studies only evaluated the safety concerning hepatotoxicity of antifungal medication in mostly non-ICU

Table 3. Liver enzymes and function

A	Anidulafungin (n=30)	Micafungin (n=33)	P
Liver failure*	9 (30)	2 (6)	0.01
Increased serum AST (U/L) after start*	13 (43)	12 (36)	0.58
Increased serum ALT (U/L) after start*	13 (43)	10 (30)	0.29
Serum AST (U/L) at start	95 (282)	55 (76)	0.09
Serum ALT (U/L) at start	56 (125)	39 (59)	0.13
Serum AST (U/L) at stop	115 (1011)	52 (108)	0.17
Serum ALT (U/L) at stop	75 (312)	45 (68)	0.14
Serum AST (U/L) peak	285 (228)	116 (181)	0.11
Serum ALT (U/L) peak	150 (404)	70 (126)	0.15
Serum bilirubin (µmol/L) peak	71 (192)	15 (29)	0.05
Prothrombin Time (sec) peak	18.3 (15.4)	17.8 (8.0)	0.27
B	**Anidulafungin (n=21)**	**Micafungin (n=31)**	**P**
Increased serum AST (U/L) after start*	8 (38)	11 (35)	0.85
Increased serum ALT (U/L) after start*	7 (33)	9 (29)	0.74
Serum AST (U/L) at start	61 (98)	55 (73)	0.82
Serum ALT (U/L) at start	43 (75)	39 (54)	0.89
Serum AST (U/L) at stop	44 (141)	49 (79)	0.98
Serum ALT (U/L) at stop	41 (83)	45 (54)	0.99
Serum AST (U/L) peak	129 (267)	113 (158)	0.88
Serum ALT (U/L) peak	69 (209)	61 (119)	0.95
Serum bilirubin (µmol/L) peak	20 (105)	14 (27)	0.74
Prothrombin Time (sec) peak	16.6 (7.5)	17.8 (5.3)	0.68

Numbers (percentage)* or median (interquartile range). Liver enzymes and function in all patients who were treated with anidulafungin and micafungin (A) and in patients without liver failure (B). Abbreviations: ALT = alanine transaminase; AST = aspartate transaminase.

Table 4. Treatment costs

Anidulafungin	Micafungin	
	(n=30)	(n=33)
Total treatment days	354	429
Total drug dose(mg)	41.600	42.900
Total drug costs (€)	183,805.44	209,553.63
Drug costs per patient (€)	6,126.84	6,350.11
Drug costs p.p.p.d. (€)	519.22	488.47

Micafungin costs € 460.82 (excluding VAT) per 100 mg ampoule, € 488.47 (including 6% VAT). Anidulafungin costs € 416.83 (excluding VAT) per 100 mg ampoule, € 441.84 (including 6% VAT). Abbreviations: p.p.p.d. = per patient per day.

patients, but did not evaluate mortality or efficacy, as we did. Therefore, this study adds important information about the safety and efficacy of micafungin compared with anidulafungin in critically ill patients. At day 28 the overall mortality was 60% which is comparable to the mortality described in other studies [30,31]. The data suggest that higher 90-day mortality with anidulafungin than micafungin reflects more severe underlying liver disease rather than effect of treatment itself.

The type and duration of treatment of invasive candidiasis depends on culture results and sensitivity testing, the extent of organ involvement and patients' clinical condition [9,24]. In patients with invasive fungal infections the recommended length of therapy is 14 days after the first negative blood culture [9,24], which is in line with the observed median length of treatment of 12-14 days in this study. The overall response rates in this study are comparable to those found in other performed studies [11,32]. The overall response rate of micafungin is comparable to that of anidulafungin. Our retrospective data, concerning the safety and efficacy of micafungin are in line with a recent performed study, which evaluated the safety and efficacy of micafungin monotherapy in critically ill patients with cancer and invasive candidiasis [32]. However, the study did not make a comparison with anidulafungin, as we did. The pharmacokinetics of micafungin are very well defined in non-critically ill patients and seems to be similar in critically ill patients [33]. In critically ill patients micafungin reaches a steady state by day 3, without the need for a loading dose, in contrast to caspofungin and anidulafungin [9,24, 25,33]. Dose adaptations are not required for body weight and in patients with renal or hepatic impairment and renal replacement therapy [33]. The involved costs per patient per day of treatment with micafungin seems to be a bit lower compared with anidulafungin.

One of the limitations of this study is the relatively small number of patients included, so that results should be regarded as preliminary. This study enrolled 63 patients with invasive candidiasis caused by *Candida spp*. in a four year period in a tertiary care ICU with 2,000 admissions per year. Hence, the reported incidence of invasive candidiasis in a large review was 5 to 10 cases per 1,000 ICU admission, which is comparable with our reported incidence of 6 per 1,000 ICU admissions [34]. Second, because of the retrospective design we cannot exclude that the use of either agent may have been subject to bias.

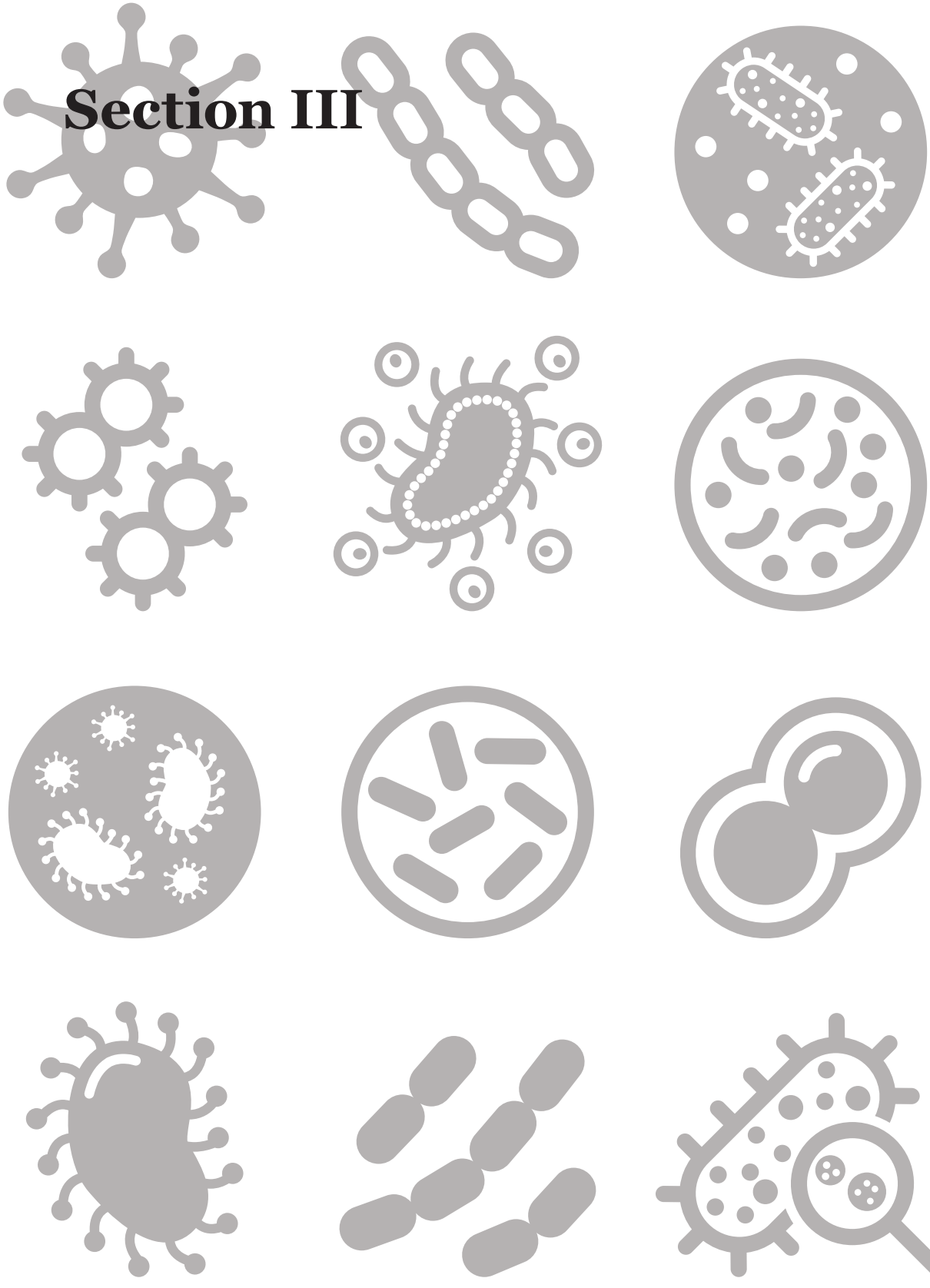
In conclusion, our results suggest that micafungin can be safely and effectively used in critically ill patients with candidemia and invasive candidiasis.

References

1. Kett DH, Azoulay E, Echeverria PM, Vincent JL. Candida bloodstream infections in intensive care units: analysis of the extended prevalence of infection in intensive care unit study. Crit Care Med 2001; 39: 665-670.
2. Glockner A. Treatment and prophylaxis of invasive candidiasis with anidulafungin, caspofungin and micafungin – review of the literature. Eur J Med Res 2011; 16: 167-179.
3. Pelz RK, Lipsett PA, Swoboda SM, Diener-West M, Hammond JM, Hendrix CW. The diagnostic value of fungal surveillance cultures in critically ill patients. Surg Infect 2000; 1: 273-281.
4. Dimopoulos G, Ntziora F, Rachiotis G, Armaganidis A, Falagas ME. Candida Albicans versus non-candida albicans intensive care unit-acquired bloodstream infections: differences in risk factors and outcome. Anesth Analg 2008; 106: 523-529.
5. Prowle GR, Echeverri JE, Ligabo EV, Sherry N, Taori GC, Crozier TM, et al. Acquired bloodstream infection in the intensive care unit: incidence and attributable mortality. Crit Care 2011; 15: R100.
6. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of candida bloodstream infection until positive bloodculture results are obtained: a potential risk factor for hospital mortality. Antimicrob Agents Chemother 2005; 49: 3640-3645.
7. Garey KW, Rege M, Pai MP, Mingo DE, Suda KJ, Turpin RS, et al. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi institutional study. Clin Infect Dis 2006; 45: 25-31.
8. Pfaller M, Boyken I, Hollis RJ, Kroeger J, Messer SA, Tendolkar S, et al. In vitro susceptibility of invasive isolates of candida spp. to anidulafungin, caspofungin, and micafungin: six years of global surveillance. J. Clin Microbiol 2008; 46: 150-156.
9. Pappas PG, Kauffman CA, Andes D, Benjamine DK Jr, Calandra TF, Edwards JE Jr, et al. Clinical guidelines for the management of candidiasis: 2009 update by the infectious diseases society of America. Clin Infect Dis 2009; 48: 505-535.
10. Van Burik JH, Ratanatharathorn V, Stepan DE, Miller CB, Lipton JH, Vesole DH, et al. Micafungin versus fluconazole for prophylaxis against invasive fungal infections during neutropenia in patient undergoing hematopoietic stem cell transplantation. Clin Infect Dis 2004; 39: 1407-1416.
11. Reboli AC, Rotstein CM, Pappas PG, Chapman SW, Kett DH, Kumar D, et al. Anidulafungin versus fluconazole for invasive candidiasis. N Engl J Med 2007; 356: 2472-2482.
12. Hiramatsu Y, Maeda Y, Fuji N, Saito T, Nawa Y, Hara M, et al. Use of micafungin versus fluconazole for antifungal prophylaxis in neutropenic patients receiving hematopoietic stem cell transplantation. Int J Hematol 2008; 88: 588-595.
13. Guery BP, Arendrup MC, Auzinger G, Azoulay E, Borges Sá M, Johnson EM, et al. Management of invasive candidiasis and candidemia in adult non-neutropenic intensive care unit patients: Part II treatment. Intensive Care Med 2009; 35: 206-214.
14. Marchetti O, Eggimann P, Calandra T. Invasive candidiasis in critically ill patients: does progressing knowledge improve clinical management and outcome? Curr Opin Crit Care 2010; 16: 442-444.
15. Kett DH, Shorr AF, Reboli AC, Reisman AL, Biswas P, Schlamm HT. Anidulafungin compared with fluconazole in severely ill patients with candidemia and other forms of invasive candidiasis: support for the 2009 IDSA treatment guidelines for candidiasis. Crit Care 2011; 15: R253.
16. Pappas PG, Coleman M, Rotstein F, Nucci M, Talwar D, De Waele JJ, et al. Micafungin versus caspofungin for treatment of candidemia and other forms of invasive candidiasis. Clin Infect Dis 2007; 45: 883-893.
17. Dimopoulos G, Antonopoulou A, Armaganidis A, Vincent JL. How to select an antifungal agents in critically ill patients. J Crit Care 2013; 28: 717-727.

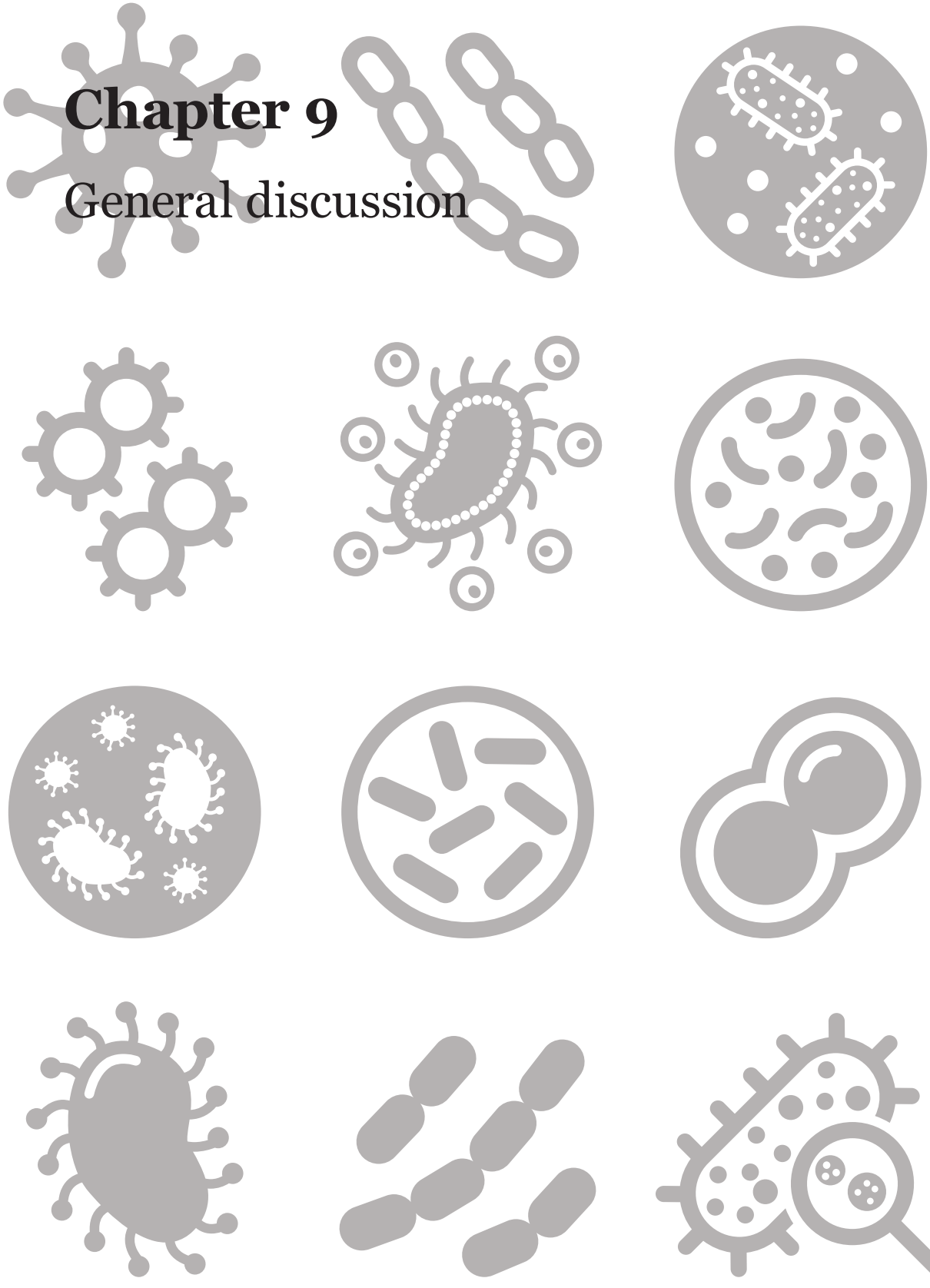
18. Kubiak DW, Bryar JM, McDonnell AM, Delgado-Flores JO, Mui E, Baden LR, et al. Evaluation of caspofungin or micafungin as empiric antifungal therapy in adult patients with persistent febrile neutropenia: a retrospective, observational, sequential cohort analysis. Clin Ther 2010; 32: 637-648.
19. Kohno S, Izumikawa K, Yoshida M, Takesue Y, Oka S, Kamei K, et al. A double-blind comparative study of the safety and efficacy of caspofungin versus micafungin in the treatment of candidiasis and aspergillosis. Eur J Clin Microbial Infect Dis 2013; 32: 387-397.
20. Wang JL, Chang CH, Young-Xu Y, Chan KA. Systematic review and meta-analysis of the tolerability and hepatotoxicity of antifungals in empirical and definitive therapy for invasive fungal infection. Antimicrob Agents Chemother 2010; 54: 2409-2419.
21. Schneeweiss S, Galar A, carver P, Johnson M, Marty F, Nagel J, et al. Multicenter cohort study of the safety of Micafungin and other parenteral antifungals. ICAAC-ICC 2015; poster presentation M-364.
22. Bassetti M, Marchetti M, Chakrabarti A, Colizza S, Garnacho-Montero J, Kett DH, et al. A research agenda on the management of intra-abdominal candidiasis: results from a consensus of multinational experts. Intensive Care Med 2013; 39: 2092-2106.
23. Vazquez J, Reboli AC, Pappas PG, Patterson TF, Reinhardt J, Chin-Hong P, et al. Evaluation of an early step-down strategy from intravenous anidulafungin to oral azole therapy for the treatment of candidemia and other forms of invasive candidiasis: results from an open-label trial. BMC Inf Dis 2014; 14: 97.
24. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortolary O, et al. ESCMID guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. Clin Microbiol Inf 2012; 18: 19-37.
25. Oude Lashof AML, Janssen JJWM, Meis JFGM, Warris A, Wout JW van 't, Natsch SS, et al. SWAB richtlijn voor de behandeling van invasieve schimmelinfecties 2013. NVMM 2013; 18:13-17.
26. León C, Ruiz-Santana S, Saavedra P, Galván B. Blanco A, Castro C, et al. Usefulness of the 'Candida score' for discriminating between Candida colonization and invasive candidiasis in non-neutropenic critically ill patients: A prospective multicenter study. Crit Care Med 2009; 37: 367 1624-1633.
27. Kullberg BJ, Verweij PE, Akova M, Arendrup MC, Bille J, Calandra T, et al. European expert opinion on the management of invasive candidiasis in adults. Clin Microbial Inf 2011; 5: 1-12.
28. Cappelletty D, Eiselstein-McKitrick K. The echinocandins. Pharmacotherapy 2007; 27: 369-88.
29. Thomson SJ, Cowan L, Johnston I, et al. Liver function tests on the intensive care unit: a prospective, observational study. Intensive Care Med 2009; 35: 1406-1411.
30. Guery BP, Arendrup MC, Auzinger G, Azoulay E, Borges Sa M, Johnson EM, et al. Management of invasive candidiasis and candidemia in adult non-neutropenic intensive care unit patients: Part I. Epidemiology and diagnosis. Intensive Care Med 2009; 35: 55-62.
31. Leroy O, Gangneux JP, Montravers P, Mira JP, Gouin F, Sollet JP, et al. AmarCand study group. Epidemiology, management, and risk factors for death of invasive Candida infections in critical care: a multicenter, prospective, observational study in France (2005-2006). Crit Care Med 2009; 309 37: 1612-8..
32. Farmakiotis D, Tarrand JJ, Kontoyiannis DP. Efficacy and tolerability of micafungin monotherapy for candidemia and deep-seated candidiasis in adults with cancer. Antimicrob Agents Chemother 2014; 58: 3526-3529.
33. Lempers VJ, Schouten JA, Hunfeld NG, Colbers A, Van Leeuwen HJ, Burger DM, et al. Altered micafungin pharmacokinetics in intensive care unit patients. Antimicrob Agents Chemother 2015; 59: 4403-4409.
34. Eggimann P, Bille J and Marchetti O. Diagnosis of invasive candidiasis in the ICU. Ann Intensive Care 2011; 1: 37.

Section III



Chapter 9

General discussion



General discussion

The research questions we posed in the general introduction of this thesis have been answered. However, to come to a well-balanced conclusion the results of the different articles in chapter 2 till 5 needs to be combined.

Like the introduction of this thesis, we will also divide this discussion into two sections. Section I of this discussion will discuss the results of the four articles on different biomarkers for the prediction of infection and it's sequelae. Section II of this discussion will discuss the results of the three articles on the safety and efficacy of different antifungals.

Section I - Prediction of infection in the critically ill

In most clinics the white blood cell count (WBC) and C-reactive protein (CRP) are used as markers for the prediction of infection in critically ill patients [1]. Less frequently procalcitonin (PCT) is used as well. These biomarkers have been in use for decades and seem to be useful in daily clinical practice, though, sensitivity and specificity of these markers are suboptimal, which results in moderate operational characteristics at best [1].

To impact the attributable morbidity and mortality levels it is necessary to diagnose infections in an early stage [2-7]. Despite many years of research and development of new biomarkers for infection, diagnosing infection continuous to be a challenge and the quest for new and better biomarkers continuous [1]. More than 170 biomarkers, as possible new markers for detection of infection, have been studied over the last decades. One of those markers is the immature granulocyte, as discussed in Chapter 2 of this thesis. The host response to a microbial infection invariably starts with the activation of the innate immune system [8]. Consequently, it is reasonable to focus on cell specific haematological information and methods aimed at detecting this early response to systemic infection [8]. Plasma cells or lymphoplasmocytoid cells are involved in the first line defence against pathogens [9]. In addition to the activation of circulating immune cells, the bone marrow responds to systemic infection as well by releasing immature granulocytes into the peripheral blood [10]. Two previous studies showed that the presence of immature granulocytes in critically ill patients predict the presence of infections better than CRP does [11,12]. However the

additive predictive value of immature granulocytes for serious infections (i.e. bacteraemia) remained unclear [12-14]. In Chapter 2 of this thesis we showed that the predictive value for infection of immature granulocytes is better than the WBC, but equal to CRP. For bacteraemia, the predictive value of immature granulocytes was better than that of WBC or CRP. Although this new marker looks promising, its AUROC for the prediction of infection was 0.73 with corresponding sensitivity of 58% and specificity of 80% at a cut-off value of 0.4%. Although an AUROC >0.70 is often considered as clinical relevant [15], the AUROC value of the immature granulocytes is just an fraction above this cut-off value, and as a result of the low sensitivity a large number of infections will still be missed. Thus, immature granulocytes cannot be used as a single parameter to rule out infection. As mentioned in the introduction section of this thesis, it will be unlikely that a single parameter will have sufficiently power for the prediction of infection, since sepsis is the result of a very complex host immune response to infection [1].

Combining several parameters into a scoring system for the prediction of infection could be a possible option to improve the operational characteristics for the prediction of infection [1]. Indeed, combining different markers of infection led to better predictive values, as shown in Chapter 2 and 5 of this thesis. In Chapter 2 we combined the immature granulocytes with WBC and CRP and described the predictive value for infection for this combination of markers. The addition of immature granulocytes to WBC and CRP resulted in a better predictive value as compared to using WBC or CRP as single parameter, even when CRP and WBC were used together. The question arises which combination of markers should be used to optimize the diagnostic accuracy for the prediction of infection. Another question is whether we should use markers of infection only or that we should implement clinical variables (e.g. heart rate, respiratory rate) or clinical scores (e.g. SOFA, APACHE) as well. Peres Bota et al. devised the Infection Probability Score which combines clinical parameters (temperature, heart rate, respiratory rate, SOFA score) as well as biomarkers (WBC, CRP) to predict infection [16]. Another scoring system by Lukaszewski et al. only used biomarkers (IL-1β, -6, -8, -10, TNF-α, FasL and CCL2 mRNA) [17]. Although both scoring systems look promising, there is a substantial difference in the predictive values for both scoring systems. Peres et al. described a positive predictive value of 53.6% and Lukaszewski et al. described a predictive value of

83% [16,17]. In Chapter 3 of this thesis we also describe a novel scoring system for the prediction of infection. The scoring system called, The Intensive Care Infection Score (ICIS), uses 5 blood cell derived parameters for the prediction of infection. All these five blood cell derived parameters of the ICIS score characterize the early innate immune respons, so this infection score focuses at the early response to systemic infection [8]. The predictive value of ICIS was indeed better than that of WBC, CRP or PCT. Though the predictive value of ICIS was not superior compared to CRP and PCT. In fact, the predictive value of ICIS was comparable to that of the immature granulocytes, as described in Chapter 2 of this thesis. Although positive predictive values of the different infection scoring systems vary substantially, using these scoring systems for the prediction of infection seem to be promising and useful. Further research and development of the different scoring systems is recommended, with particular attention to which combination of clinical and non-clinical parameters should be used to optimize the diagnostic accuracy for the prediction of infection.

So far, the positive predictive values of the different biomarkers remain insufficient to accurately predict the presence of infection. However, some of the described biomarkers seem to have a high negative predictive value. Instead of using biomarkers to predict the presence of infection, it would be more realistic to use a biomarker to rule out infection, by using its high negative predictive value. One of the biomarkers that could be used to rule out infection is procalcitonin. Previous studies demonstrated that PCT can accurately predict bacteraemia in patients with pneumoniae and fever and that bacteraemia becomes very unlikely when PCT levels are below a certain treshold [18-20]. In Chapter 4 and 5 of this thesis we focus on the diagnostic accuracy of procalcitonin for serious infections i.e. bacteraemia. In Chapter 4 we demonstrated that procalcitonin has a positive predictive value of 88% to predict bacteraemia in critically ill patients, with corresponding sensitivity of 89% and specificity of 78% at a cut-off of 0.25 ng/mL The negative predictive value of procalcitonin varied from 95-98% which makes PCT a good biomarker to rule out bacteraemia in critically ill patients. Indeed, in chapter 5 of this thesis we demonstrated in a prospective randomized controlled trial that PCT is useful to rule out bacteraemia. Ruling out infection at an early stage, in a patient suspected of having infection, is very useful. In case of a low procalcitonin (<0.25), bacteraemia is very unlikely and antibiotics can be withheld, while further exams focus on other causes of systemic inflam-

mation that may need to be addressed. In case of an elevated procalcitonin level (>0.25), bacteraemia could be possible and could guide the physician to start empiric antibiotics and strive for improved source control, in anticipation of further exams such as blood cultures or results of other biomarkers.

Future perspectives

Critical care medicine is a fairly new field of patient care. It all started with Florence Nightingale who suggested in the 1850s that critically ill patients needed special, separate care. However, it was not until some 100 years later that the first ICUs began to be built. Since those early days, intensive care medicine has grown exponentially in terms of number of patients admitted, our understanding of the complex pathophysiological processes affecting ICU patients, and technological developments [21-23]. In Europe over five million adults are admitted to an ICU every year. As the population ages, more patients are treated with immunosuppressive therapies, and the boundaries of medical treatment continue to expand, this number will continue to rise [21-23]. Moreover, as emergency care transport and management improves, more patients will survive acute illness and severe trauma to reach the ICU [21-23]. As a results of all those factors it will be likely that the incidence of sepsis will continue to increase as well [21-23]. For the clinician the future challenge would be to correctly discriminate infectious from non- infectious causes of systemic inflammation. Therefore it's crucial that the search for better biomarkers for the prediction of infection continuous, where the focus should be on further improving the above described infection scores. Other more recent techniques (proteomics, genomics and PCR techniques) are very complex and it remains to be seen if they will become part of our diagnostic armamentarium [1]. Special attention should be paid to the combination of parameters to be used in a new scoring system. Maybe the clinicians clinical judgement should be added to a scoring system, which is, according to my view, still a fast and reliable decision tool to decide whether a patient is suffering from an infection or not. Further research should also focus on the use of the negative predictive value of biomarkers to rule out infection and the consequences of excluding an infection. In case an infection is very unlikely blood cultures can be withheld and antibiotics are unnecessary. In addition, further research

is needed to better define the role of biomarkers when deciding to continue or stop antibiotic treatment in a patient with an infection. A recently performed study showed promising results in the reduction and consumption of antibiotics in critically ill patients by using a procalcitonin guided algorithm [24]. This will result in lower costs for the intensive care unit, but more important is that the patient will not be unduly exposed to antibiotics. These efficient strategies which could lead to a reduction of antibiotic prescription are urgently needed, because of the ever increasing bacterial resistance to multiple antimicrobial agents [25].

Section II – Candida infections in the critically ill

Over the last decades substantial progress has been made on the diagnostic and therapeutic arsenal to conquer infection, but still infectious complications continue to be a major challenge in critical care medicine [26]. In critically ill patients the incidence of invasive fungal infections has increased remarkably as a result of the widespread use of broad spectrum antibiotics, advanced care in the intensive care unit, the ever increasing patient population on immunosuppressive therapy and improved knowledge on fungal infections [26-28].

For the management of invasive candidiasis there is no single strategy that can be considered the most appropriate [29]. In particular, four management options are available: prophylaxis, empirical therapy, pre-emptive therapy, and treatment of a culture proven infection [29]. In this thesis we focus on the treatment of culture proven invasive candidiasis in critically ill patients, as well as on prophylaxis in patients colonized with *Candida* spp.. During the last decades several new antifungal drugs haven been developed. The choice of a particular antifungal drug is based on local and international guidelines, the aim of therapy, the properties of the drug and local epidemiology [30-32]. Local and international guidelines give recommendations on the optimal length of treatment and the possibility to step down to smaller spectrum drugs [30-32]. The evidence in favor of or against a particular antifungal treatment strategy is often scarce.

In Chapter 6 of this thesis we evaluated the safety and efficacy of amphotericin-B deoxycholate inhalation therapy for the treatment of *Candida* spp. colonization of the respiratory tract. Amphotericin-B is part of selective

decontamination of the digestive tract (SDD) protocols, which were introduced in 1983 in the Netherlands. Even though amphotericin-B is used for many years in critically ill patients to eradicate Candida spp. from the respiratory tract, little is known about its safety and efficacy [33]. The use of inhaled amphotericin-B deoxycholate has been associated with a decreased activity of pulmonary surfactant in rats [34-36]. In humans, liposomal amphotericin-B inhalation may not alter surfactant but may be cytotoxic by other means [37-38]. In Chapter 6 we showed that the use of conventional amphotericin-B deoxycholate was associated with a longer length of mechanical ventilation, independent of the presence of ventilator associated pneumonia. Patients using amphotericin-B remained mechanically ventilated for an additional 13 days, with higher lung injury scores and low P_aO_2/F_IO_2 ratios in this group. The decreased activity of pulmonary surfactant caused by amphotericin- B deoxycholate results from the direct damage to the alveolar capillary membrane, resulting in an influx of surfactant-inactivating plasma proteins [34-35]. Surfactant is important to stabilize the alveoli at low lung volumes and to prevent alveolar collapse during physiological conditions and in acute lung injury [38]. Alveolar collapse results in more inhomogeneous ventilation which is correlated with increased risk of ventilator associated lung injury, as demonstrated by Blankman et al [39]. Loss of surfactant could therefore lead to longer requirement of mechanical ventilation in patients receiving inhalational amphotericin- B deoxycholate. Furthermore, phospholipids and proteins of pulmonary surfactant provided an important component for the innate immune defence mechanism of the lung [38]. We can speculate that an impaired innate immune defence results in a higher risk for pulmonary infections. However, an impaired surfactant activity has not been associated with the use of liposomal amphotericin- B and may therefore be a patient-friendlier alternative [34,35,40].

As said before, amphotericin-B is part of the SDD protocol which was introduced in 1983. Since then no studies have been performed to evaluate the safety of this strategy. In 2013 Ong et al. performed the first cohort trial to evaluate the efficacy of amphotericin-B nebulization therapy in critically ill patient with Candida spp. colonization of the respiratory tract [33]. They concluded that amphotericin-B facilitated faster decolonization of the respiratory tract as compared to patients who did not receive amphotericin-B. However, effective decolonization of the respiratory tract did not results in a decrease in the incidence

of ventilator associated pneumonia, ICU length of stay or mortality. Therefore they do not recommend to use amphotericin-B as part of the SDD protocol for decolonization of the respiratory tract [33]. In contrast to the faster decolonization as seen by Ong et al, in Chapter 6 of this thesis we could not confirm that the use of amphotericin-B deoxycholate facilitates a faster decolonization of the respiratory tract. Furthermore we demonstrated that the use of amphotericin-B deoxycholate is associated with an increased length of mechanical ventilation. We therefore do not recommend to use amphotericin-B deoxycholate to achieve decolonization of the respiratory tract in critically ill patients, as its use is not effective and is associated with pulmonary toxicity.

The safety and efficacy of certain antifungals is not well established in critically ill patients, because only a small fraction of the patients included in phase III trials were admitted to the ICU. In addition, also the safety and efficacy of certain antifungal treatment strategies are not well described. In Chapter 7 of this thesis we evaluated the safety and efficacy of a echinocandin to fluconazole step-down therapy in patients with invasive infections caused by *C. albicans* who were initially treated with an echinocandin. In critically ill patients with a proven invasive fungal infection, guidelines recommend to start with an echinocandin [31,41]. Step down to oral fluconazole is possible in patient who have improved clinically, and in whom fluconazole susceptible *Candida spp*. is the cause of the infection [31,41]. However, some experts recommend to continue antifungal treatment with an echinocandin [44,43]. At this moment experts and guidelines differ on the recommendations on the exact time to apply a step-down approach. Some experts opt to step-down at day 5 of treatment in patients who have clinically improved after initial treatment with an echinocandin, while other guidelines prefer to step-down at day 10 of treatment [31,41,44,45]. In Chapter 7 of this thesis we showed that applying an early step-down strategy can be done at day 4 after start of echinocandin therapy, in critically ill patients with candidemia or invasive candidiasis caused by fluconazole susceptible *C. albicans* who have clinically improved. We showed that applying an early step-down strategy can be safely applied without an increased mortality rate in the early step-down group. Our results are comparable to those in other studies and with the recent AmarCAND2 study in France [45-47]. Acquired resistance to echinocandins, caused by FKS mutations have been described recently and typically occur after very prolonged treatment of critically ill patients with echi-

nocandins [48,49]. Applying a step-down strategy to fluconazole decreases the echinocandin exposure time and may partially prevent the occurrence of these mutations and the risk for breakthrough infections with echinocandin resistant *Candida* spp. [50]. Furthermore, substantial cost savings are another advantage of step down-therapy [51]. In conclusion, we recommend to apply an early step-down to fluconazole in critically ill patients with invasive fungal infections susceptible for fluconazole, who have clinically improved after initial treatment with an echinocandin. This step-down can already be performed at day 4.

The last part of this discussion will focus on the safety and efficacy of micafungin for the treatment of invasive fungal infection in critically ill patients. Micafungin is one of the three echinocandins, which are part of a relatively novel class of antifungal agents. Caspofungin was introduced in 2001 for the treatment of invasive fungal infections, followed by anidulafungin and micafungin respectively [52,53]. Echinocandins inhibit the beta-1,3-D-glucan synthase enzyme complex, which causes loss of resistance to osmotic forces and cell lysis among *Candida* spp. and are therefore fungicidal [53]. The intracellular beta-glucan synthase complex is not present in human cells. Therefore the echinocandins cause less toxicity than amphotericin-B or triazoles and few drug-drug interactions are reported [53]. The major advantages of echinocandins relative to other antifungal agents are their fungicidal activity against *Candida* spp, including fluconazole-resistant *C. glabrata* and *C. krusei*, combined with their relatively low potential for renal or hepatic toxicity or serious drug-drug interactions [53-55]. Most experts consider all three echinocandins to be interchangeable for the treatment of invasive candidiasis, since these drugs have a rather similar in vitro spectrum of activity and mechanism of action [53-56]. Though, most intensivists prefer anidulafungin above micafungin for the treatment of invasive candidiasis in patients with severe liver injury or liver failure, as micafungin has been associated with hepatotoxicity in some studies [57]. However, these results were obtained from studies using high dosages for prolonged time in male animals and studies on humans or other animals data did not report similar effects [55,57] In chapter 8 of this thesis we evaluated the safety and efficacy of micafungin versus anidulafungin for the treatment of invasive fungal infections in critically ill patients. We demonstrated that the safety and efficacy of the treatment with micafungin was similar to that of anidulafungin. No differences were seen in response rates, liver function and enzymes, and mortality.

We therefore suggest that micafungin, like anidulafungin and caspofungin, can be safely and effectively used in critically ill patients with invasive fungal infections. There is no special preference for the use of a particular echinocandin for the treatment of an invasive fungal infection [54]. However, the three echinocandins differ in terms of their dosing, pathways of metabolic elimination, and drug interaction profiles [54]. Therefore, it is important to appreciate these unique characteristics when selecting an echinocandin. For example, no loading dose is needed for micafungin and no dose adaptation needs to be made for body weight and for patients with renal or hepatic impairment, in contrast to anidulafungin or micafungin [58].

Future perspective

The incidence of invasive fungal infections in critically ill patients will continue to increase during the coming years [21-24]. This is the direct consequence of an aging population, the increased number of patients treated with immunosuppressive therapies and the expanding boundaries of medical treatment [21-24]. Invasive fungal infections are associated with increased morbidity and mortality [27-28]. To reduce these levels, the efficacy and safety of the different antifungal drugs and antifungal treatment strategies need to be well established. The safety and efficacy of the different antifungal drugs and antifungal treatment strategies is not well established in critically ill patients [58]. This is the result of the very small number of patients included in most antifungal registration trials. Therefore further research is necessary to improve further understanding of the different antifungal drugs and treatment strategies, to improve survival in critically ill patients with invasive fungal infections.

With the increasing incidence of invasive fungal infections, the incidence in antifungal drug resistance in *Candida* spp. is also increasing [48]. This reflects the expanding use of newer broad spectrum antifungals [48]. Further research should therefore focus on the exact mechanisms of developing antifungal drug resistance, and methods for the rapid detection of resistance. It is also important to evaluate the usefulness of different antifungal treatment strategies, particularly in patients for whom the potential benefit of treatment is unproven. For example, a recent study showed that pre-emptive therapy with micafungin was

not effective at reducing the development of invasive candidiasis compared with placebo in critically ill patients requiring emergency gastrointestinal surgery [59,60]. Avoiding unnecessary use of antifungal drugs will partially prevent the occurrence of antifungal resistance and the risk of break through infections [48]. Further research should also focus on the development of voriconazole resistant aspergillus species [61]. With the increasing incidence of voriconazole resistant aspergillus spp., liposomal amphotericin- B may reoccur as an important treatment alternative in critically ill patients with invasive pulmonary aspergillosis.

Further research should also focus on the pharmacodynamics and pharmacokinetics of the different antifungal drugs when used in the critically ill. Over the last decade the boundaries of medical treatment in critically ill patients expanded significantly, for example with the broad implementation of extra corporeal membrane oxygenation. A better understanding of the pharmacodynamics and pharmacokinetics of the different antifungal drugs in patients exposed to new medical techniques has the potential to improve their survival.

References

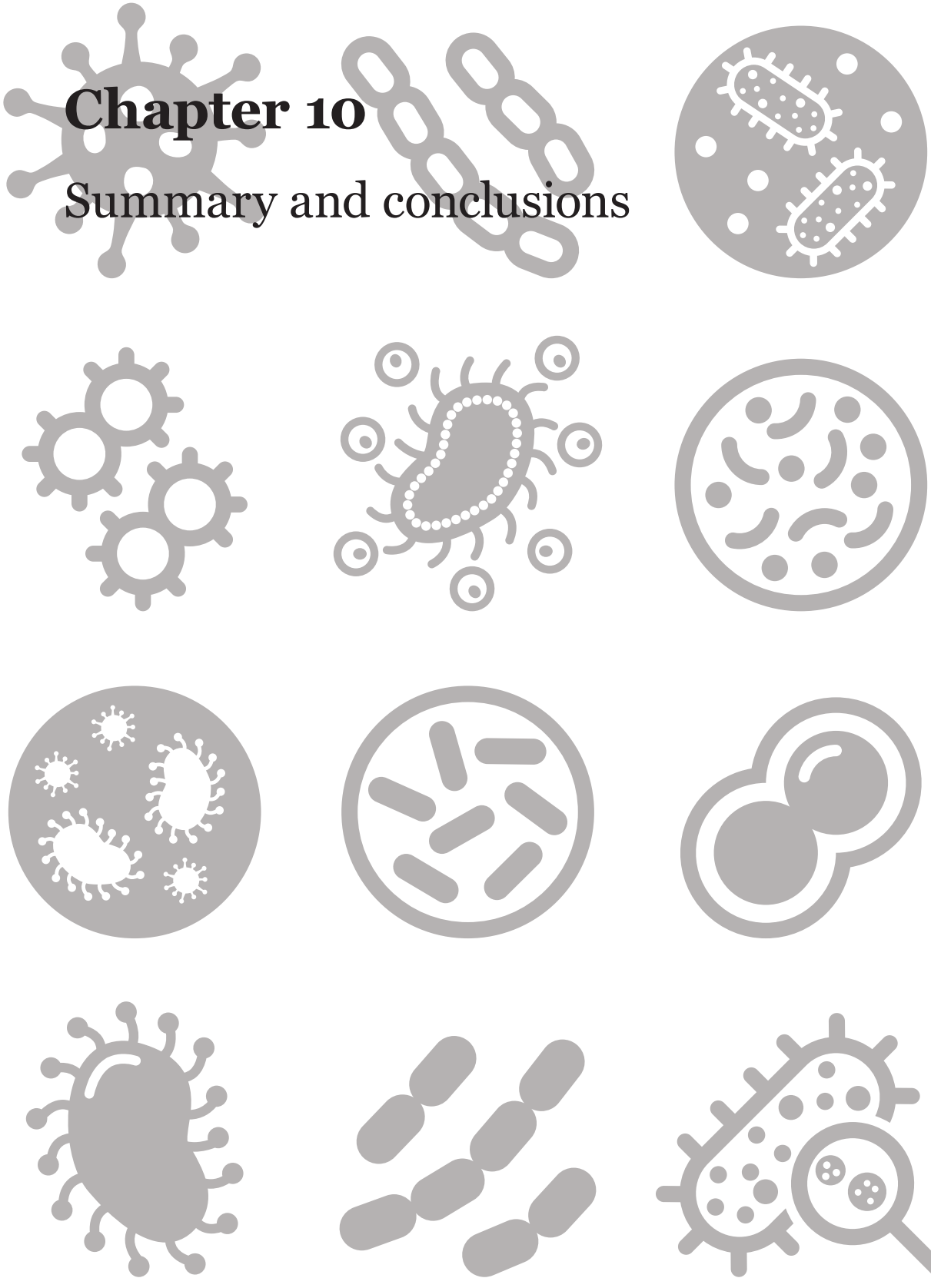
1. Vincent JL, Beumier M. Diagnostic and prognostic markers in sepsis. Expert Rev Anti Infect Ther 2013; 11: 265-275.
2. Angus DC, Linde-Zwirble WT, Lidicker J, et al. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001; 29: 1303-10.
3. Van Gestel A, Bakker J, Vervaart CP, et al. Prevalence and incidence of severe sepsis in Dutch intensive care units.
4. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006;34:1589-96.
5. Vincent JL, Sakr Y, Sprung CL, et al. Sepsis in European intensive care units: results of the SOAP study.. Crit Care Med 2006; 34: 344-53.
6. Wang HE, Szychowski JM, Griffin R, et al. Long-term mortality after community-acquired sepsis:a longitudinal population-based cohort study. BMJ Open 2014;4:e004283.
7. Goncalves-Pereira J, Pereira JM, Ribeiro O, et al. Impact of infection on admission and of the process of care on mortality of patients admitted to the Intensive Care Unit – The INFAUCI study. Clin Microbiol Infect. 2014; 20: 1308-153.
8. Smith JA. Neutrophils, host defense, and inflammation: a double-edged sword. Journal of leukocyte biology. 1994 Dec;56(6):672-86.
9. Linssen J, Jennissen V, Hildmann J, et al. Identification and quantification of high fluorescence-stained lymphocytes as antibody synthesizing/secreting cells using the automated routine hematology analyzer XE-2100. Cytometry Part B, Clinical cytometry. 2007 May;72(3):157-66.
10. Bender L, Thaarup J, Varming K, Krarup H, Ellermann-Eriksen S, Ebbesen F. Early and late markers for the detection of early-onset neonatal sepsis. Danish medical bulletin. 2008 Nov;55(4):219-23.
11. Park BH, Kang YA, Park MS et al. Delta neutrophil index as an early marker of disease severity in critically ill patients with sepsis. BMC Infect Dis 2001; 11:29.
12. Nierhaus A, Klatte S, Linssen J et al.Revisiting the white blood cell count: immature granulocytes count as a diagnostic marker to discriminate between SIRS and sepsis – a prospective, observational study. BMC Immunology 2013; 14:8.
13. Nahm CH, Choi JW, Lee J. Delta neutrophil index in automated immature granulocyte counts for assessing disease severity of patients with sepsis. Ann Clin Lab Sci 2008; 38:241-246.
14. Seok Y, Choi JR, Kim J et al. Delta neutrophil index: a promising diagnostic and prognostic marker for sepsis. Shock 2012; 37:242-246.
15. Tape TG. Interpreting diagnostic tests, http://gim.unmc.edu/dxtests/, accessed 14 June 2016
16. Pere Bota D, Melot C, Lopes Ferreira F, et al. Infection probability score (IPS): a method to help assess the probability of infection in critically ill patients. Crit Care Med 2003; 31: 2579-2584.
17. Lukaszewski RA, Yates AM, Jackson, et al. Presymptomatic prediction of sepsis in intensive care unit patients. Clin Vaccine Immunol 2008; 15: 1089-1094.
18. Muller F, Christ-Crain M, Bregenzer T, et al. Procalcitonin levels predict bacteremia in patients with community-acquired pneumonia: a prospective cohort trial. Chest 2010;138:121-9.
19. Kim MH, Lim G, Kang SY, Lee WI, Suh JT, Lee HJ. Utility of procalcitonin as an early diagnostic marker of bacteremia in patients with acute fever. Yonsei Med J 2011;52:276-81.
20. Schuetz P, Mueller B, Trampuz A. Serum procalcitonin for discrimination of blood contamination from bloodstream infection due to coagulase-negative staphylococci. Infection 2007;35:352-5.
21. Vincent JL, Singer M. Critical care: advances and future perspectives. Lancet 2010; 376: 1354-1361.
22. Vincent JL, Singer M, Marini JJ, et al. Thirty years of critical care medicine. Crit Care 2010; 14: 311.
23. Vincent JL. Critical Care – Where have we been and where are we going? Crit Care 2013; 17: S2.

24. De Jong E, Van Oers JA, Beishuizen A, et al. Efficacy and safety of procalcitonin guidance in reducing the duration of antibiotic treatment in critically ill patients: a randomised, controlled, open-label trial. Lancet Infect Dis 2016; 16: 819-827.
25. S.E. Cosgrove. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. Clin Infect Dis 2006; 42; 82–89.
26. Elhoufi A, Ahmadi A, Asnaashari AMH, et al. Invasive candidiasis in critical care setting, updated recommendations from "Invasive Fungal Infections-Clinical Forum", Iran. World J Crit Care Med 2012; 3: 102-112.
27. Kett DH, Azoulay E, Echeverria PM, Vincent JL. Candida bloodstream infections in intensive care units: analysis of the extended prevalence of infection in intensive care unit study. Crit Care Med 2001; 39: 665-670
28. Glockner A. Treatment and prophylaxis of invasive candidiasis with anidulafungin, caspofungin and micafungin – review of the literature. Eur J Med Res 2011; 16: 167-179.
29. Bassetti M, Mikulska M and Viscoli C. Bench-to-bedside review: Therapeutic management of invasive candidiasis in the intensive care unit. Critical care 2010; 14: 244.
30. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 2009; 48: 503-535.
31. Cornely OA, Bassetti M, Calandra T, et al. ESCMID guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. Clin Microbiol Inf 2012; 18: 19-37.
32. Oude Lashof AML, Janssen JJWM, Meis JFGM, et al. SWAB- richtlijn voor de behandeling van invasieve schimmelinfecties 2013. NVMM 2013; 18: 13-17.
33. Ong DS, Klein Klouwenberg P, Spitoni C, Bonten MJ, Cremer OL. Nebulised amphotericin B to eradicate Candida colonisation from the respiratory tract in critically ill patients receiving selective digestive decontamination: a cohort study. Crit Care 2013; 17: R233.
34. Van 't Veen A, Gommers D, Mouton JW, Kluytmans JA, Krijt EJ, Lachmann B. Exogenous pulmonary surfactant as a drug delivering agent: influence of antibiotics on surfactant activity. Br J Pharmacol 1996; 118: 593-598.
35. Ruijgrok EJ, Vulto AG, Van Etten WM. Efficacy of aerosolized amphotericin B desoxycholate and liposomal amphotericin B in the treatment of invasive pulmonary aspergillosis in severely immunocompromized rats. J Antimicrob Chemother 2001; 48: 89-95.
36. Knechtel SA, Klepser ME. Safety of aerolized amphotericin B. Expert Opin Drug Saf 2007; 6: 523-532.
37. Fauvel M, Farrugia C, Tsapis N, et al. Aerosolized liposomal amphotericin B: prediction of lung deposition, in vitro uptake and cytotoxicity. Int J Pharm 2012; 436: 106-110.
38. Monforte V, López-Sánchez A, Zurbano F, et al. Prophylaxis with nebulized liposomal amphotericin B for Aspergillus infection in lung transplant patients does not cause changes in the lipid content of pulmonary surfactant. J Heart Lung Transplant 2013; 32: 313-319.
39. Blankman P, Shono A, Hermans BJ, Wesselus T, Hasan D, Gommers D. Detection of optimal PEEP for equal distribution of tidal volume by volumetric capnography and electrical impedance tomography during decreasing levels of PEEP in post cardiac-surgery patients. Br J Anaesth 2016; 116: 862-9.
40. Khan MA and Owais M. Toxicity, stability and pharmacokinetics of amphotericin B in immunomodulatory tuftsin-bearing liposomes I a murine model. J. Antimicrob. Chemother 2006; 58: 125-132.
41. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 2009; 48: 503-535.
42. Colombo AL, Guimaraes T, Sukienik T, et al. Prognostic factors and historical trends in the epidemiology of candidemia in critically ill patients: an analysis of five multicenter studies sequentially conducted over a 9-year period. Intensive Care med 2014; 40: 1489-1498.

43. Kett DH, Shorr AF, Reboli AC, Reisman AL, Biswas P, Schlamm HT. Anidulafungin compared with fluconazole in severely ill patients with candidemia and other forms of invasive candidiasis: support for the 2009 IDSA treatment guidelines for candidiasis. Crit Care 2009; 15: R253.
44. Bassetti M, Marchetti M, Chakrabarti A, et al. A research agenda on the management of intra-abdominal candidiasis: results from a consensus of multinational experts. Intensive Care Med 2013; 39: 2092-2106.
45. Vazquez J, Reboli AC, Pappas PG, et al. Evaluation of an early step-down strategy from intravenous anidulafungin to oral azole therapy for the treatment of candidemia and other forms of invasive candidiasis: results from an open-label trial. BMC Inf Dis 2014; 14: 97.
46. Bal AM, Shankland GS, Scott G, Imtiaz T, Macaulay R, McGill M. Antifungal step-down therapy based on hospital intravenous to oral switch policy and susceptibility testing in adult patients with candidaemia: a single centre experience. Int J Clin Pract 2014; 68: 20-27.
47. Bally S, Leroy O, Montravers P, et al. Antifungal de-escalation was not associated with adverse outcome in critically ill patients treated for invasive candidiasis: post hoc analyses of the AmarCAND2 study data. Intensive Care Med 2015;41:1931-1940.
48. Maubon D, Garnaud C, Calandra T, Sanglard D, Cornet M. Resistance of Candida spp. To antifungal drugs in the ICU: where are we now? Intensive Care Med 2014; 40: 1241-1255
49. Alexander BD, Johnson MD, Pfeiffer CD, et al. Increasing echinocandin resistance in *Candida glabrata:* clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. Clin Infect Dis 2013; 56: 1724-1732.
50. Pfeiffer CD, Garcia-Effron G, Zaas AK, Perfect JR, Perlin DS, Alexander BD. Breakthrough invasive candidiasis in patients on micafungin. J Clin Microbiol 2010; 48: 2373-2380.
51. Bal AM, Shankland GS, Scott G, Imtiaz T, Macaulay R, McGill M. Antifungal step-down therapy based on hospital intravenous to oral switch policy and susceptibility testing in adult patients with candidaemia: a single centre experience. Int J Clin Pract 2014; 68: 20-27.
52. Pfaller MA, Boyken L, Hollis RJ, Messer SA, Tendolkar S and Diekema DJ. In vitro susceptibilities of *Candida* spp. to Caspofungin: four years of global surveillance. J. Clin. Microbiol 2006; 44: 3533-8.
53. Pfaller M, Boyken I, Hollis RJ, et al. In vitro susceptibility of invasive isolates of candida spp. to anidulafungin, caspofungin, and micafungin: six years of global surveillance. J. Clin Microbiol 2008; 46: 150-156.
54. Pappas PG, Coleman M, Rotstein F, et al. Micafungin versus caspofungin for treatment of candidemia and other forms of invasive candidiasis. Clin Infect Dis 2007; 45: 883-893.
55. Dimopoulos G, Antonopoulou A, Armaganidis A, Vincent JL. How to select an antifungal agents in critically ill patients. J Crit Care 2013; 28: 717-727.
56. Pfaller MA, Rhomberg PR, Messer SA, et al. Isavuconazole, micafungin, and 8 comparator antifungal agents' susceptibility profiles for common and uncommon opportunistic fungi collected in 2013: temporal analysis of antifungal drug resistance using CLSI species-specific clinical breakpoints and proposed epidemiological cutoff values. Diagn Microbiol Infect Dis 2015; 82:303.
57. Glockner A. Treatment and prophylaxis of invasive candidiasis with anidulafungin, caspofungin and micafungin – review of the literature. Eur J Med Res 2011; 16: 167-179.
58. Lempers VJ, Schouten JA, Hunfeld NG, et al. Altered micafungin pharmacokinetics in intensive care unit patients. Antimicrob Agents Chemother 2015; 59: 4403-4409.
59. Knitsch W, Vincent JL, Utzolino S, et al. A randomized, placebo-controlled trial of preemptive antifungal therapy for the prevention of invasive candidiasis following gastrointestinal surgery for intra-abdominal infections. Clin Infect Dis. 2015;61:1671–8.
60. Calandra T, Roberts JA, Antonelli M, Bassetti M and Vincent JL. Diagnosis and management of invasive candidiasis in the ICU: an updated approach to an old enemy. Crit Care 2016; 20: 125.
61. Verweij PE. Azole resistance in *Aspergillus fumigatus*. In: NethMap 2015. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. The Hague: National Institute for Public Health and the Environment; 2015. p. 113–5.

Chapter 10

Summary and conclusions



Introduction

In the first part of this thesis we evaluated the diagnostic accuracy of biomarkers for the prediction of serious infections in critically ill patients. The main goal was to study the predictive properties of different biomarkers in critically ill patients, to evaluate the effectiveness of combining biomarkers into a diagnostic score, and to discuss the limitations for their applicability in clinical practice. In the second part of this thesis we evaluate the safety and efficacy of different antifungal treatment strategies and antifungal medication.

Section I - Prediction of infection in the critically ill

In Chapter 2 of this thesis we describe the predictive value of immature granulocytes percentage (IG%) in comparison with the white blood cell count (WBC) and C-reactive protein (CRP), for the prediction of infection, bloodstream infection, and septic shock in critically ill patients. Blood samples (IG%, WBC, CRP) were collected in 46 consecutive patients at the day (0) of a clinical suspicion of microbial infection and at day 1 and 3 thereafter. A clinical suspicion of infection could be raised by the presence of a body temperature >38 °C or <36 °C measured rectally while in the ICU, a WBC count >10,000/μL or <4,000/μL or CRP >9 mg/L. In patients suspected for infection microbial cultures were taken. We only considered infections confirmed by a positive microbial culture as primary endpoint (i.e. proven infection). Thirty-one patients had a proven infection, of which 13 patients developed septic shock. Bacteraemia was confirmed in 15 patients. Our results suggest that IG% is a useful marker, as CRP, for the prediction of infection, its invasiveness and severity in a critically ill patient suspected for having infection.

Although the results of the IG% seems to be promising, it will be unlikely that this single parameter will be used for the prediction of infection, since sepsis is the result of a complex host immune response to infection. An option would be to combine different biomarkers, whether or not with clinical data, into a scoring system. Indeed, the diagnostic accuracy for the prediction of infection increased when IG%, WBC and CRP were used together, as shown in Chapter 2. In Chapter 3 we evaluate the diagnostic accuracy of a novel diagnostic score, the intensive care infection score (ICIS) for the prediction of infection, and

compared its performance with WBC, CRP and procalcitonin (PCT). The ICIS consists of five blood cell-derived parameters characterizing the early innate immune response and routinely obtainable in blood samples sent to the laboratory for cell counts.. We prospectively obtained 301 blood samples in patients suspected for having an infection. Patients were divided into groups of increasing likelihood of infection and invasiveness, suggestive of increasing severity: Group 1 without infection or with possible infection irrespective of cultures, Group 2 with probable (irrespective of cultures) or proven local infection (with positive cultures of a causative microorganism) without BSI and Group 3 with BSI irrespective of local infection. The results suggest that this novel diagnostic score is potentially useful for the prediction of infection in critically ill patients suspected for having infection. However, the predictive properties of ICIS did not significantly differ from that of CRP or PCT. Further research and development of the different scoring systems is recommended, with particular attention to which combination of clinical and non-clinical parameters should be used to optimize the diagnostic accuracy for the prediction of infection.

One of the most studied biomarkers for the prediction of infection is PCT. Though, the results of the different studies on the diagnostic use of PCT have been contradicting. This could be explained by the fact that in the majority of the literature PCT is used to diagnose sepsis rather than proven microbial infection. In this thesis we have tried to study the use of biomarkers for the prediction of serious infections, i.e. bacteraemia, which is a more robustly defined culture proven infection. In Chapter 4 we performed a systematic review and meta-analysis to study the diagnostic accuracy of PCT for bacteraemia. The study included 58 articles which described 16,514 patients of whom 3.420 suffered from bacteraemia. The results showed that PCT had a fair diagnostic accuracy for the prediction of bacteraemia in adult patients suspected for sepsis or infection. At a cut-off value of 0.5 ng/mL the area under the hierarchical summary receiver operating characteristic (HSROC) was 0.79, with corresponding sensitivity of 76% and specificity of 69%. In particular low procalcitonin levels can be used to rule out the presence of bacteraemia, because of its high negative predictive value. This could lead to a cost reduction by saving blood cultures.

In Chapter 5 of this thesis we studied the safety and efficacy of PCT of guiding blood culture taking in critically ill patients with suspected infection. We performed a prospective multi-center, cluster randomized, cross-over trial

in 564 clinical patients suspected for having infection. Patients were divided in a control group (standard of care) and a PCT-guided group. In both groups blood was drawn at the same moment for a PCT measurement and blood culture.

In the PCT-guided group blood cultures were not sent if the PCT was below 0.25 ng/ml, unless otherwise indicated. The control and intervention group included 288 and 276 patient respectively. Eighteen sets of blood cultures were saved in 17 patients in the PCT- guided group. There was no difference in mortality at day 28 and day 90 between both groups. Using this strategy could save 1.14 euro per suspected infection episode. The results of this study suggest that PCT to guide blood cultures in critically ill patients with suspected infection can be used safely and (cost-)effectively.

Section II – Candida infection in the critically ill

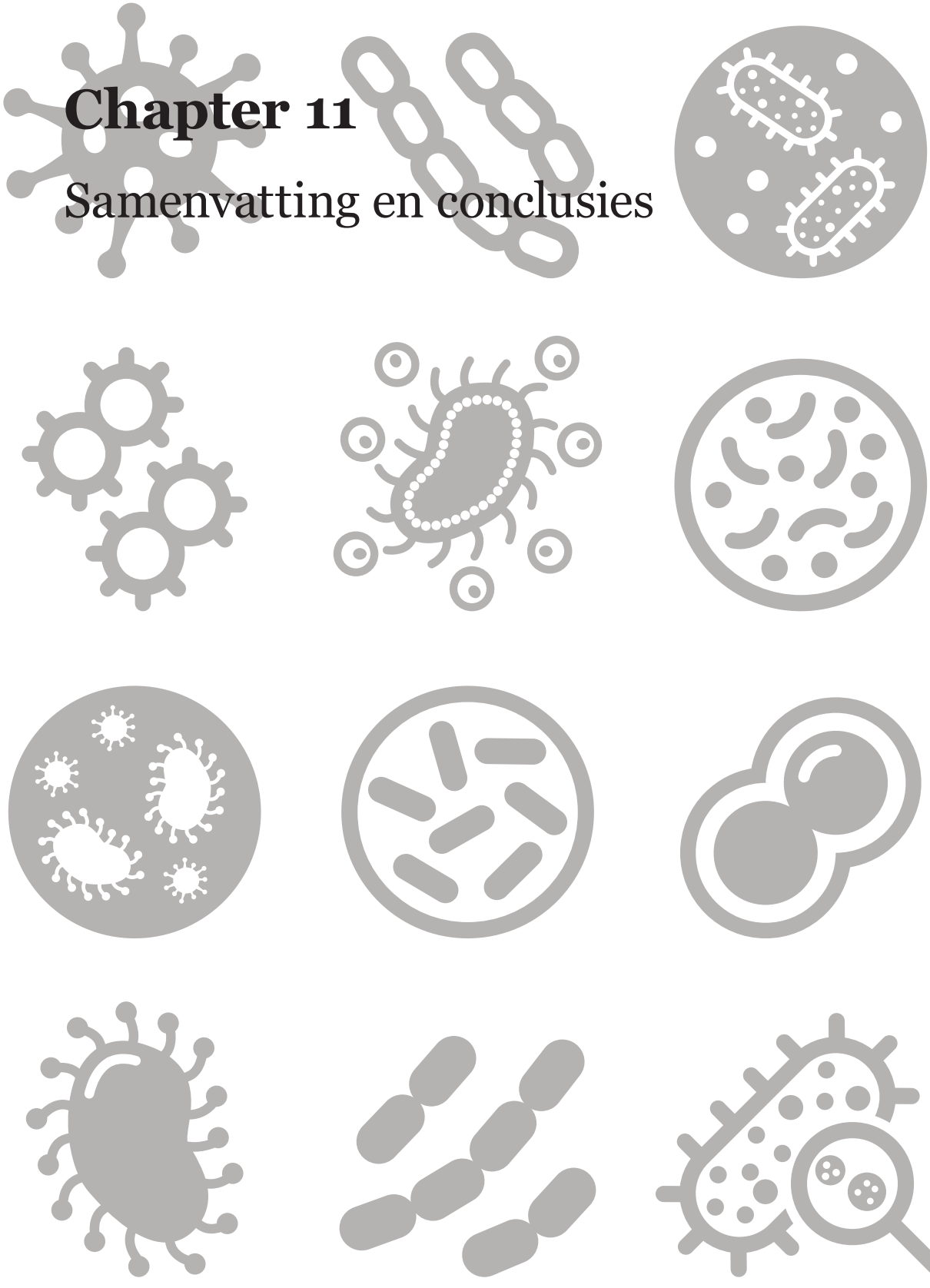
In section II of this thesis we focus on the antifungal treatment of invasive *Candida* infections in te critically ill patient. Selective decontamination of the digestive tract (SDD) is a frequently used strategy in many Dutch intensive care units (ICU's) and consists of oral administration of non-absorbable antibiotics. Amphotericin-B deoxycholate (ABDC) inhalation therapy is started via a nebulizer is case of respiratory colonization with *Candida* spp. This treatment is started as part of many SDD protocols used in large trials.. However, its safety and efficacy are not well established, in the absence of randomized trials. In Chapter 6 of this thesis we performed a retrospective study to assess the safety and efficacy of ABDC inhalation for the treatment of respiratory *Candida* spp. colonization in critically ill patients. We included 113 patients who had positive *Candida* spp. cultures of the respiratory tract for more than 1 day and required mechanical ventilation >48 hours. Fifty-one of them received ABDC inhalation therapy. The results showed that using ABDC inhalation therapy does not lead to faster decolonization of the respiratory tract as in untreated patients. Furthermore, ABDC inhalation therapy is associated with an increased length of mechanical ventilation, because of direct toxicity of the drug on the lung. Therefore we do not recommend to use ABDC inhalation therapy for respiratory colonization with *Candida* spp. in non-neutropenic critically ill patients.

In critically ill patients with a proven invasive infection caused by *Candida* spp., the initial antifungal drug of choice is an echinocandin. Step-down therapy to fluconazole is advised for patients who have improved clinically after initial therapy with an echinocandin and in whom fluconazole susceptible *Candida* spp. have been documented can step- down to fluconazole. Though the exact timing to step-down is a subject of debate. We therefore performed a retrospective study in 56 patients with invasive *C. albicans* infections who initially received an echinocandin for 4 or more days (Chapter 7). Of those patients, 32 received step-down fluconazole therapy, at median day 5, whereas the echinocandin was continued in the other 24. Our results showed that the global response rates at the end of treatment, which was defined as resolution of signs and symptoms of *C. albicans* infection, were similar between the groups. No difference were seen in 28-day or 90-day mortality between the groups. Our results suggests that step-down therapy to fluconazole can be safely and effectively applied in critically ill patients with invasive, fluconazole-susceptible *C. albicans* infections, who have clinically improved after initial treatment with an echinocandin. This step-down approach can already be done at day 5 of treatment.

In the last chapter of this thesis, Chapter 8, we studied the safety and efficacy of micafungin in critically ill patients with invasive candidiasis. Micafungin has been associated with hepatotoxicity, therefore intensivists prefer anidulafungin above micafungin for the treatment of invasive fungal infections in critically ill patients with severe liver injury or liver failure. We performed a retrospective study in 63 critically ill patients with invasive fungal infections to evaluate the safety and efficacy of micafungin compared to anidulafungin. Thirty patients received anidulafungin and 33 patients received micafungin. None of the patients developed impaired liver function related to antifungal use and no differences were seen in prothrombin time, serum transaminases and bilirubin levels between the groups. The global response rates at the end of treatment, which was defined as resolution of signs and symptoms of an invasive fungal infection, was similar for both groups. No difference was seen in 28-day mortality, but 90-day mortality was higher in patients on anidulafungin. The increased 90-day mortality was not correlated with the use of anidulafungin, as shown in the cox regression analysis. Our results suggest that micafungin can be effectively used in critically ill patient with invasive candidiasis and is not associated with hepatotoxicity.

Chapter 11

Samenvatting en conclusies



Introductie

In het eerste deel van dit proefschrift onderzochten we de diagnostische waarde van biomarkers voor de voorspelling van ernstige infecties bij ernstig zieke patiënten. Het belangrijkste doel was om de voorspellende eigenschappen van de verschillende biomarkers te bestuderen bij ernstig zieke patiënten , de effectiviteit van het combineren van biomarkers in een diagnostische score te evalueren, en om de beperkingen van hun toepasbaarheid in de klinische praktijk te bespreken. In het tweede deel van dit proefschrift evalueerden we de veiligheid en effectiviteit van verschillende antifungale behandelingsstrategieën en antifungale medicatie.

Deel I – Voorspellen van infectie in de ernstig zieke patiënt

In hoofdstuk 2 van dit proefschrift beschrijven we de voorspellende waarde van het percentage onrijpe granulocyten (IG%), vergeleken met witte bloedcellen (WBC) en C-reactief proteïne (CRP), voor het voorspellen van infectie, bloedvergiftiging en shock in de ernstig zieke patiënt. In 46 patiënten die verdacht werden van infectie werd bloed afgenomen voor het bepalen van IG%, WBC en CRP. Bloed werd afgenomen op dag 0 (dag van inclusie) infectie en op dag 1 en 3 daarna. De klinische verdenking op een infectie kan worden ondersteund door de aanwezigheid van een lichaamstemperatuur > 38 ° C of <36 ° C rectaal gemeten, een WBC telling> 10.000 / ul of <4000 / ul of een CRP> 9 mg / l . Er werden kweken afgenomen van patiënten die verdacht werden van een infectie. Het primaire eindpunt in deze studie was een bewezen infectie middels een positieve kweek. In totaal hadden 31 patiënten een bewezen infectie, waarvan 13 patiënten in shock waren. In 5 patiënten was er sprake van een bloedvergiftiging. De resultaten van onze studie suggereren dat IG%, net als CRP, een bruikbare marker is voor het voorspellen van infectie, de invasiviteit van de infectie en de ernst van een infectie in een ernstig zieke patiënt.

Ondanks de veelbelovende resultaten van IG%, is het onwaarschijnlijk dat een enkele biomarker gebruikt zal worden voor het voorspellen van infectie, omdat sepsis het resultaat is van een complexe reactie van het lichaam op een infectie. Het combineren van verschillende biomarkers in een scoresysteem

zou een oplossing kunne vormen voor dit probleem. In hoofdstuk 2 hebben we eveneens IG%, WBC en CRP samengevoegd tot een scoresysteem, waarmee we aantonen dat de voorspellende waarde voor infectie verbetert. In hoofdstuk 3 hebben we een nieuw scoresysteem geëvalueerd, de intensive care infectie score (ICIS), voor het voorspellen van infectie, waarbij we de voorspellende waarde van ICIS hebben vergeleken met die van WBC, CRP en PCT. ICIS bestaat uit een vijftal cellijnen welke betrokken zijn bij de eerste respons van het lichaam op een infectie. De gegevens voor deze studie hebben we prospectief verzameld in 301 patiënten die werden verdacht voor het hebben van een infectie. Patiënten werden ingedeeld in verschillenden groepen van toenemende invasiviteit en ernst van de infectie. Groep 1 bevatte patiënten zonder infectie of met een mogelijke infectie. Groep 2 bevatte patiënten met een bewezen infectie of een zeer waarschijnlijke infectie. Tot slot bevatte groep 3 patiënten met een bewezen infectie en daarbij een bloedvergiftiging. De resultaten van het onderzoek suggereren dat ICIS een potentieel bruikbaar scoresysteem is voor het voorspellen van infecties in de ernstig zieke patiënt. Echter, de voorspellende waarde voor infectie van ICIS was niet significant beter dan die van CRP of PCT. Het wordt dan ook aanbevolen om verder onderzoek te verrichten naar de ontwikkelingen van verschillende scoresystemen. Hierbij moet vooral worden gelet op welke combinatie van klinische en niet- klinische parameters gebruikt moet worden om de diagnostische nauwkeurigheid voor de voorspelling van infectie te optimaliseren.

PCT is een van de meest onderzochte biomarkers voor de voorspelling van infectie. De resultaten van de verschillende onderzoeken naar het gebruik van PCT voor het voorspellen van infectie zijn echter tegenstrijdig. Dit kan worden verklaard door het feit dat in de meeste literatuur PCT wordt gebruikt voor het voorspellen van sepsis in plaats van een bewezen microbiële infectie. Daarom hebben wij ervoor gekozen in dit proefschrift om biomarkers te gebruiken voor het voorspellen van ernstige infecties, zoals bijvoorbeeld een bloedvergiftiging, welke een bewezen microbiële infectie is. Hoofdstuk 4 beschrijft de resultaten van een systematische review en meta-analyse naar de nauwkeurigheid va PCT voor het voorspellen van bloedvergiftiging. De studie omvatte 58 artikelen met 16.514 patiënten, waarvan 3.420 patiënten een bloedvergiftiging hadden. De resultaten toonden aan dat PCT een goede voorspellende waarde had voor het voorspellen van bloedvergiftiging in ernstig zieke patiënten. Bij een afkapwaarde

van 0,5 ng/ml was de hiërarchische summary receiver operating characteristic (HSROC) 0,79, met overeenkomstige sensitiviteit van 76% en specificiteit van 69%. Vanwege de hoge negatieve voorspellende waarde, kunnen lage PCT-waarden gebruikt worden voor het uitsluiten van een bloedvergiftiging. Dit resulteert in een kostenbesparing door verminderde afname van bloedkweken.

In hoofdstuk 5 van dit proefschrift bestudeerden we de veiligheid en effectiviteit van PCT geleide bloedkweek afname bij ernstig zieke patiënten met een vermoedelijke infectie. Hiervoor hebben we een prospectief multi-center, cluster gerandomiseerd, cross-over onderzoek verricht bij 564 klinische patiënten die werden verdacht van het hebben van een infectie. De patiënten werden verdeeld in een controlegroep (standaardbehandeling) en een PCT-geleide groep. In beide groepen werd bloed afgenomen op hetzelfde moment voor een PCT bepaling en een bloedkweek. In de PCT-geleide groep werden bloedkweken niet verzonden indien de PCT waarde lager was dan 0,25 ng / ml, tenzij anders aangegeven. In totaal werden 288 patiënten geïncludeerd in de controlegroep en 276 patiënten in de interventiegroep. In de PCT-geleide groep werden 18 bloedkweken bespaard in 17 patiënten. Er was geen verschil in mortaliteit op dag 28 en dag 90 tussen beide groepen. Met behulp van deze strategie kan 1,14 euro bespaard worden per vermoedelijke infectie episode. De resultaten van deze studie suggereren dat een PCT -geleide bloedkweek afname, veilig en (kosten)effectief gebruikt kan worden in ernstige zieke patiënten, die worden verdacht van een infectie.

Deel II – Candida infecties in de ernstig zieke patiënt

In deel II van dit proefschrift richtten we ons op de behandeling van invasieve Candida-infecties in de ernstig zieke patiënt. Selectieve de contaminatie van het spijsverteringskanaal (SDD) is een veel gebruikte strategie in veel Nederlandse intensive care units (ICU) en bestaat uit orale toediening van niet-absorbeerbare antibiotica. Amfotericine-B deoxycholaat (ABDC) inhalatie therapie wordt gestart via een verstuiver indien er sprake is van kolonisatie van de luchtwegen met *Candida* spp. Deze behandeling wordt gestart als onderdeel van vele SDD protocollen, welke uitgebreid zijn onderzocht in diverse onderzoeken. Echter, de veiligheid en effectiviteit van ABDC inhalatietherapie is niet goed onderzocht. In

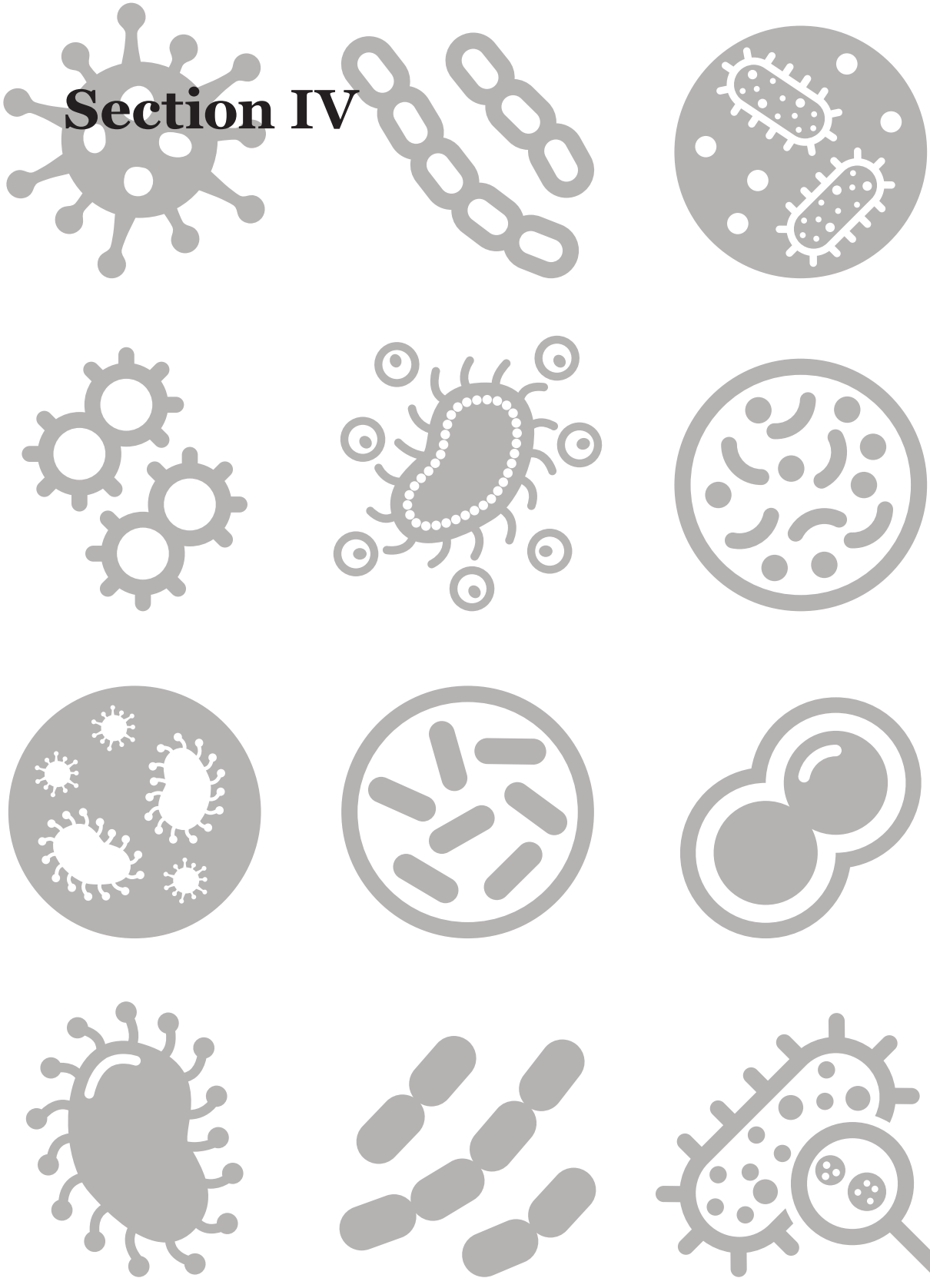
hoofdstuk 6 van dit proefschrift hebben we een retrospectieve studie verricht naar de veiligheid en werkzaamheid van ABDC inhalatietherapie voor de behandeling van respiratoire *Candida* spp. kolonisatie bij ernstig zieke patiënten. In totaal werden 113 patiënten geïncludeerd met positieve keelkweken voor *Candida* spp. en die langer dan 48 uur mechanisch werden beademd. Eenenvijftig patiënten werden behandeld met ABDC inhalatie therapie. De resultaten toonde aan dat het gebruik van ABDC inhalatietherapie niet leidt tot snellere dekolonisatie van de luchtwegen. Daarnaast werd ABDC inhalatietherapie geassocieerd met een toegenomen lengte van mechanische beademing, als gevolg van directe toxiciteit van het geneesmiddel op de long. Daarom raden we niet aan om ABDC inhalatietherapie te gebruiken voor dekolonisatie van *Candida* spp. in de luchtwegen.

Ernstig zieke patiënten met bewezen invasieve *Candida* spp. infectie worden bij voorkeur behandeld met een echinocandine. Therapieversmalling naar fluconazol wordt geadviseerd voor patiënten die klinisch zijn verbeterd na initiële behandeling met een echinocandine en bij wie er sprake is van een fluconazol-gevoelige *Candida* spp. De exacte timing om therapieversmalling toe te passen blijft een onderwerp van discussie. Daarom hebben wij een retrospectieve studie verricht in 56 patiënten met invasieve *C. albicans* infecties, waarbij de initiële behandeling uit een echinocandine bestond, voor een periode van tenminste vier dagen (hoofdstuk 7). In totaal werd therapieversmalling naar fluconazol toegepast in 32 van de 56 patiënten. Dit gebeurde gemiddeld op dag 5 van behandeling. De overige 24 patiënten werden uitbehandeld met de echinocandine. Onze resultaten lieten zien dat beide behandelstrategieën even effectief waren. Er was geen verschil in sterfte tussen beide groepen op dag 28 of 90.

Onze resultaten suggereren dat therapieversmalling naar fluconazol, na initiële behandeling met een echinocandine, veilig en effectief kan worden toegepast in ernstige zieke patiënten met een bewezen *C. albicans* infectie, welke gevoelig is voor fluconazol. Onze resultaten suggereren dat therapieversmalling naar fluconazol veilig en effectief kan worden toegepast in ernstig zieke patiënten met invasieve fluconazol-gevoelige *C. albicans* infecties, die klinisch zijn verbeterd na initiële behandeling met een echinocandine. De therapieversmalling kan al worden toegepast op dag 5 van de behandeling.

In het laatste hoofdstuk van dit proefschrift, hoofdstuk 8, hebben we de veiligheid en effectiviteit van micafungin bij ernstig zieke patiënten met invasieve candida infecties onderzocht. Micafungin is geassocieerd met leverschade, waardoor intensivisten de voorkeur geven aan anidulafungin boven micafungin voor de behandeling van invasieve candida infecties bij ernstig zieke patiënten met ernstige leverbeschadiging of leverfalen. We hebben een retrospectieve studie verricht in 63 ernstig zieke patiënten met invasieve candida infecties om de veiligheid en effectiviteit van micafungin, vergeleken met anidulafungin, te evalueren. Dertig patiënten kregen anidulafungin en 33 patiënten kregen micafungin. Geen van de patiënten ontwikkelde leverfunctiestoornissen of levertestafwijkingen ten gevolge van de antifungale medicijnen. Het succes van de behandeling was gelijk voor beide groepen. Er was geen verschil in sterfte tussen beide groepen op dag 28. Echter, de sterfte op dag 90 was hoger voor patiënten die werden behandeld met anidulafungin. Na het verrichten van een Cox-regressie analyse kwam naar voren dat de verhoogde sterfte op dag 90 niet werd veroorzaakt door anidulafungin, maar werd veroorzaakt door de onderliggende ziekte bij de patiënt Onze resultaten suggereren dat micafungin effectief kan worden gebruikt in ernstig zieke patiënten met invasieve candida infecties en er geen associatie is met het optreden van leverschade.

Section IV



List of Authors and Affiliations

Rob Brouwer
Department of Clinical Chemistry
Erasmus University Medical Center
Rotterdam, The Netherlands

Erik I. Dieters
Department of Intensive Care Medicine
Erasmus University Medical Center
Rotterdam, The Netherlands

Servet Duran
Department of Intensive Care Medicine
Maasstad Ziekenhuis
Rotterdam, The Netherlands

Patrick J. van der Geest
Department of Intensive Care Medicine
Erasmus University Medical Center
Rotterdam, The Netherlands

A.B. Johan Groeneveld
Department of Intensive Care Medicine
Erasmus University Medical Center
Rotterdam, The Netherlands

Sandra H. Hoeboer
Department of Internal Medicine
VU Medical Center
Amsterdam, The Netherlands

Ben van den Hoven
Department of Intensive Care Medicine
Erasmus University Medical Center
Rotterdam, The Netherlands

Nicole G.M. Hunfeld
Department of Farmacie and Intensive Care
Erasmus University Medical Center
Rotterdam, The Netherlands

Robert de Jonge
Department of Clinical Chemistry
VU Medical Center
Amsterdam, The Netherlands

Jo Linssen
Department of Immunology
University of Herdecke/ Witten
Herdecke/ Witten, Germany

Mostafa Mohseni
Department Of Intensive Care Medicine
Erasmus University Medical Center
Rotterdam, The Netherlands

Daan Nieboer
Department of Public Health
Erasmus University Medical Center
Rotterdam, The Netherlands

Bart J.A. Rijnders
Department of Internal Medicine and infectious diseases
Erasmus University Medical Center
Rotterdam, The Netherlands

Ewout W. Steyerberg
Department of Public Health
Erasmus University Medical Center
Rotterdam, The Netherlands

Alieke G. Vonk
Department of Medical Microbiology
Erasmus University Medical Center
Rotterdam, The Netherlands

Publications related to this thesis

1. Van der Geest PJ, Mohseni M, Brouwer R, Van der Hoven B, Steyerberg E, Groeneveld ABJ: Immature granulocytes predict microbial infection and its adverse sequelae in the intensive care unit. Journal of Critical Care 2014; 29: 523-7.
2. Van der Geest PJ, Dieters EI, Rijnders B, Groeneveld ABJ: Safety and efficacy of amphotericin-B deoxycholate inhalation in critically ill patients with respiratory Candida spp. colonization: a retrospective analysis. BMC Infectious Diseases 2014; 14: 575.
3. Van der Geest PJ, Hoeboer SH, Groeneveld ABJ: The diagnostic accuracy of procalcitonin for bacteremia: a systematic review and meta-analysis. Clinical Microbiology and Infection 2015; 21: 474-81.
4. Van der Geest PJ, Rijnders B and Groeneveld ABJ. Echinocandin to fluconazole step-down therapy in critically ill patients with invasive, susceptible Candida albicans infections. Mycoses 2016; 59: 179-85.
5. Van der Geest PJ, Mohseni M, Linssen J, Duran S, De Jonge R and Groeneveld ABJ. The Intensive Care Infection Score – A promising marker for the prediction of infection and its severity. Critical care 2016; 20: 180.
6. Van der Geest PJ, Hunfeld NGM, Ladage SE, Rijnders BJA and Groeneveld ABJ. Micafungin versus anidulafungin in critically ill patients with invasive candidiasis: a retrospective study. BMC infectious disease. 2016; 16: 490.
7. Van der Geest PJ, Mohseni M, Nieboer D, Duran S and Groeneveld ABJ. Procalcitonin to guide taking bloodcultures in the intensive care unit. Clinical Microbiology and Infection.

Publications not related to this thesis

1. Van Lier F, Schouten PO, Van der Geest PJ, et. all.: Effect of chronic Beta-Blocker use on stroke after noncardiac surgery. The American Journal of Cardiology 2009; 104(3): 429-33.
2. Van Lier F, Van der Geest PJ, Hoeks SE, et all.: Epidural analgesia is associated with improved health outcomes of surgical patients with chronic

obstructive pulmonary disease. The American Journal of Anesthesiology 2011; 115(2): 315-21.

3. Van Lier F, Van der Geest PJ,_Hol JW, Leebeek FW, Hoeks SE: Risk modification for postoperative pulmonary embolism; timing of postoperative prophylaxis. Thrombosis and Research 2012; 129(4): e14-7.
4. Van der Geest PJ, Van der Jagt M, Van Thiel R, Van Bommel J: ARDS with refractory hypoxemia and severe septic shock caused by melioidosis: Successful treatment with veno venous extra corporeal membrane oxygenation. Anesthesiology and Intensive Care 2013; 41(2): 262-3.

Presentations

1. Immature granulocytes predict microbial infection and its adverse sequelae in the intensive care unit. Van der Geest PJ, Mohseni M, Brouwer R, Van der Hoven B, Steyerberg E, Groeneveld ABJ. Annual Congress of the European Society of Intensive Care Medicine, Paris, 2013.
2. Safety and efficacy of amphotericin-B deoxycholate inhalation in critically ill patients with respiratory Candida spp. colonization: a retrospective analysis. Van der Geest PJ, Dieters EI, Rijnders B, Groeneveld ABJ. International symposium on Intensive Care and Emergency Medicine, Brussels, 2014.
3. The Intensive Care Infection Score – A promising marker for the prediction of infection and its severity. Van der Geest PJ, Mohseni M, Linssen J, Duran S, De Jonge R, Groeneveld ABJ. International symposium on Intensive Care and Emergency Medicine, Brussels, 2016.
4. Biomarkers for the prediction of sepsis. Van der Geest PJ. Sysmex European Hematology Symposium, Istanbul, 2016.

PhD Portfolio

1. PhD training	Year	Workload (ECTS)
General courses		
- Basic introduction course on SPSS		1.0
- Basis regelgeving onderzoek (BROK)		1.5
- Biomedical Scientific English writing course		2.0
- Research management for PhD students		1.0
In-depth COEUR courses		
- Intensive Care research		2.0
International conferences – participation and presentation		
- ESICM 2013, Paris, France, poster presentation	2013	1.4
- ISICEM 2014, Brussels, Belgium, poster presentation	2014	1.4
- ISICEM 2016, Brussels, Belgium, poster presentation	2016	1.4
- Sysmex European Symposium, Istanbul, oral presentation	2016	1.4
Seminars and workshops		
- Intensive Care Adults research meetings (weekly)	2011-2015	2.0
- Journal Club Intensive Care (weekly)	2011-2014	2.0
- Various Intensive Care (evening) symposia	2011-2014	2.0

2. Teaching	Year	Workload (ECTS)
Supervising Master's theses		
- Co- supervision of E.I. Dieters	2014	1.0
- Co- supervision of S. Ladage	2014	1.0
- Co- supervision of M. Mohseni	2014-2015	2.0
- Co-supervision of G. Turgut	2015	1.0
Staff member external board of reviewers EJM	2014-2015	1.0
Supervising practical's		
- Various classes of intensive care minor students	2012-2014	3.0
- Various classes of intensive care master students	2012-2014	1.0

ECTS: European Credit Transfer and Accumulation System. 1 ECTS credit represents 28 hours.

COEUR: Cardiovascular Research School Erasmus University Rotterdam.

ESICM: Annual Congress of the European Society of Intensive Care Medicine.

ISICEM: International symposium on the Intensive Care and Emergency Medicine.

Curriculum vitae

Patrick Johannes van der Geest was born on 3 august 1987 in Rotterdam, The Netherlands. After graduating from secondary school (VWO, Comenius College Capelle aan den IJssel) he studied Medicine between 2005 and 2011 at the Erasmus University Medical Center, Rotterdam, The Netherlands. After graduating university he started his professional career at the Erasmus Medical Center Rotterdam as a junior doctor in Intensive Care Medicine. During his work as junior intensive care doctor he started his PhD trajectory as described in this thesis at the department of Intensive Care Medicine under supervision of prof. dr. A.B.J. Groeneveld. After three years of working at the department of Intensive Care Medicine he switched for the period of one year to work as a junior doctor at the department of Cardiology at the Erasmus Medical Center. At January 2016 he started his specialty training in Internal Medicine at the Erasmus Medical Center, Rotterdam, The Netherlands. The first 16 months of this training will take place at the Havenziekenhuis, Rotterdam, The Netherlands.

Dankwoord

Inmiddels bent u aanbeland bij het meest gelezen hoofdstuk van het proefschrift. In dit dankwoord probeer ik een ieder te bedanken voor zijn of haar bijdrage aan dit proefschrift. Het dankwoord is zorgvuldig samengesteld, maar mocht ik iemand vergeten zijn dan hoop ik dat dit niet persoonlijk word opgevat.

Promotoren

Prof. dr. A.B.J. Groeneveld, beste Johan, je hebt er alles aan gedaan om aanwezig te kunnen zijn op mijn verdediging, maar helaas mocht het niet zo zijn. Ruim vier jaar geleden zijn we begonnen aan dit proefschrift, welke wij samen hebben afgerond tot een mooi einde. Je kwam toen ter tijd vers over uit het VU Medisch Centrum te Amsterdam, voor de meeste geboren en getogen Rotterdammers zou dit al een probleem opleveren. De samenwerking tussen 010 en 020 verliep echter zeer soepel. Ondanks het ziek worden wist je toch energie te vinden om mij te steunen met het afronden van dit proefschrift. Ik respecteer je doorzettingsvermogen, precisietijd en oplossingsvermogen. Op de momenten dat ik er klaar mee was kwam jij met een oplossing, gevolgd met succesvol resultaat. Gedurende de jaren heb ik veel van je geleerd, zowel uit de kliniek als op onderzoeksgebied. Met als grootste leerdoel: precisietijd, want zoals jij altijd zei 'het moet buiten gewoon precies'. Mijn hartelijke dank voor je vertrouwen, hulp en tijd die je gegeven hebt om dit proefschrift met succes af te ronden.

Prof. dr. D.A.M.P.J. Gommers, beste Diederik, gedurende mijn periode op de intensive care als onderzoeker en arts assistent stond jou deur altijd open voor raad en advies. Daar maakte ik dan ook regelmatig gebruik van. De afgelopen jaren waren leerzaam, verrijkend, leuk, maar ook vaak lastig. Maar voor die lastige momenten had jij vaak een ' to the point' oplossing. Ik wil je bedanken voor je luisterend oor en adviezen. Daarnaast wil ik je bedanken voor het vertrouwen wat je mij hebt gegeven de afgelopen jaren, met als resultaat het succesvol afronden van dit proefschrift.

Co- promotor

dr. B.J.A. Rijnders, beste Bart, langzaam en geleidelijk ben jij betrokken geraakt bij dit proefschrift. Ondanks alle bezigheden wist jij toch tijd te vinden om mij te begeleiden met het schrijven van de artikelen en vooral kritische en

goede verbeteringen aan te brengen in het geschreven werk. Het best kan je omschreven worden als een stille kracht, die veel belangrijk werk heeft verricht op de achtergrond. Ik wil je bedanken voor het vertrouwen dat je in mij stelde, zeker gezien het feit dat de meeste communicatie per e-mail of telefoon verliep. Bedankt dat je mijn co- promotor wilde zijn

Overige leden van de commissie

Beste prof. dr. Eric van Gorp, prof. dr. Jozef Kesecioglu, prof. dr. Jan Klein, prof. dr. Bart Jan Kullberg en prof. dr. Margreet Vos hartelijk dank voor jullie bereidheid om mijn proefschrift door te nemen en zitting te nemen in de commissie ingesteld voor mijn promotie.

De co- auteurs

Erik Dieters, beste Erik, allereerst wil ik je bedanken voor de prettige samenwerking en al het werk wat jij hebt gedaan. Het waren niet de leukste klusjes, maar mede dankzij jou inzet is mijn promotie succesvol afgerond. Waarschijnlijk heb ik je besmet met het Intensive Care en onderzoeks virus, want inmiddels zit je in dezelfde stoel als ik. Het wordt tijd om de Boudewijn weer eens af te sluiten, gouden tijden! Ik kies alleen zelf het bier weer uit, anders is the day after weer verloren.

Mostafa Mohseni, beste Moessie, bedankt voor alle hulp en de leuke samenwerking. We hebben samen een mooie onderzoekslijn opgezet met de ICIS studie. Het wordt tijd dat je de Ipad weer eens meeneemt want dan kunnen we weer World of War spelen.

Daan Nieboer, beste Daan, ik wil je bedanken voor alle statistische ondersteuning die je hebt geleverd voor de artikelen. Het waren niet altijd de makkelijkste vragen en opdrachten die moesten worden opgelost, maar gelukkig is alles geslaagd. Hopelijk heb je mijn e-mail bombardement niet als storend ervaren, bij vlagen was ik soms wat ongeduldig.

Ook de overige co- auteurs, Rob Brouwer, Ben van den Hoven, Nicole Hunfeld, Sophie Ladage, Ewout Steyerberg en Alieke Vonk, wil ik bedanken voor hun inzet en goede adviezen wat heeft geresulteerd in een aantal mooie artikelen.

De paranimfen

Paul Blankman, CHEEEEEEEEFFFFFFFF, waar zal ik dit ellendige stukje tekst weer beginnen. Allereerst wil ik je bedanken voor het feit dat je altijd voor mij klaar stond en daar waar nodig hulp kon geven. Hard werken, veel gezelligheid en doffe ellende, dat was de combinatie Paul en Patrick (alias Epi en lepsie) in het onderzoek hok. Het was maar goed dat ik halve wegen mijn promotie traject de luxe heb verworven over een eigen kamer met collega Hoeboer. Echter voor alles was een oplossing, dus het duurde niet lang voordat wij een nieuw gezamenlijk onderzoek hok hadden gevonden, de Albron (onder leiding van Koekie). In Albron vond minimaal een keer per dag overleg plaats en indien nodig tweemaal of meer. Dit onder genot van een KK'tje welke soms werd geüpgraded tot een KKF'je. Hier bespraken we de dagelijkse ellende, met als sport wiens artikel weer het snelst was afgewezen. Super tof dat je mijn paranimf wil zijn! Tot slot, ik kan het niet laten, de chef vocabulaire van de afgelopen vier jaar: Chef, doffe, ellende, terror, duif, hoe, ho, beun, goud, patat, pizza, pannenkoek, poffertjes, bier, jenever (alias de duikboot), koffie, bakkie, gevulde koek, flappelap, rue de bakkie, George V, Roosevelt, Argentine, Slopel, poes en last but not least Geile Gerard. Paul we did it!

Vincent Rietdijk, ouwe bier bom! Mensen we gaan even terug in de tijd, naar het jaar 2003, inmiddels alweer dertien jaar geleden. En de ellende is allemaal begonnen in Barcelona, met onze bus chauffeur 'kale Ronaldinho' en de docente maatschappijleer die jou even een nachtzoen kwam brengen. We weten allemaal hoe dat is afgelopen. We kennen elkaar alweer dertien jaar, kort samengevat: zuipen, bier, feest, bomen, berm, takeldienst, Wageningen, VerVer, zonnige donderdag ochtenden en heel veel gezelligheid. Dit alles gerelativeerd met Rotterdamse nuchterheid. Vincent, je stond altijd voor mij klaar, dag en nacht. Het is niet meer dan logisch dat jij dan ook mijn paranimf zou zijn, anders komt er niks terecht van dat feestje.' Oh Oh,' zou jij zeggen, 'het is weer goed met je'. Waarop mijn antwoord zou zijn: 'zeker weten' .

Collega onderzoekers Intensive Care en het onderzoekshok

Sandra Hoeboer, Engeltje, tsja waar zal ik beginnen. We hebben in hetzelfde schuitje gezeten. De afgelopen jaren hebben we gehuild, gevloekt, maar ook zeker veel gelachen. It was a hel of a job, but we did it. Soms hadden we een

pittig meningsverschil, maar meestal zaten we op een lijn en was het goed zaken doen. Tijd om weer eens een glaasje Chablis te drinken. Dank je wel poppedop.

Mo Egal, Yo Mo, waar moet ik beginnen bij jou. Als je een vraag of probleem hebt, vraag het aan Mo en hij komt met een oplossing. Vragen over Excel, Word, SPSS, R, instellen werk e-mail, codes voor bepaalde pakketten, Mo regelt het. Super bedankt voor alle ondersteuning en de gezellige momenten. Nog heel even doorbijten en ook jij bent bijna zover.

Ditty van Duijn, beste Ditty, hartstikke bedankt voor alle ondersteuning de afgelopen jaren. Promovoeren is niet alleen artikelen schrijven, maar ook heel veel administratie regelen. Gelukkig was jij overal van op de hoogte en kon jij goede ondersteuning en advies geven om het onderzoek uit te voeren.

Patricia Ormskerk, beste Gerard, allereerst: ik wacht samen met Paul Blankman nog steeds op een Bok biertje en een BBQ. Geduld wordt beloond zeggen ze. Patricia, ik wil je bedanken voor alle hulp en steun de afgelopen jaren bij mijn onderzoek. Daarnaast had jij altijd een luisterend oor en kon ik altijd bij je terecht om even te praten en alle stress en emoties te ventileren. Patricia super bedankt. En uh, hoe is het met geile Gerard?

Tot slot wil ik alle overige onderzoekers van de intensive care bedanken voor de prettige samenwerking en leuke momenten. Peter Somhorst, Matz Koeneman, Atsku Shono en Alex Lima, bedankt.

Stafleden Intensive Care Volwassenen

Joachim Weigel, Hond! Het is super fijn om jou als vriend te hebben. We hebben samen goede gesprekken gehad over diverse onderwerpen. Je had altijd een luisterend oor met gepast advies. Zowel privé als voor het werk. Daarnaast heb je mij geïnspireerd en enthousiast gemaakt voor de interne geneeskunde. Dank je wel mijn vriend. Wanneer gaan we weer Jack Ass kijken? En de halve marathon van 020? FEYENOORD.

Willy Thysse, Willy, ik wil je bedanken voor alle klinische ervaring die ik heb mogen opdoen onder jou supervisie. Daarnaast was het altijd fijn om de emoties van het intensive care werk te verwerken onder het genot van een cappuccino of een goed glas witte wijn. Thnx Willy.

Patricia Gerritsen, beste Patricia, bedankt voor alle gezellig diensten, etentjes en klinische lessen acute geneeskunde. Ik mis de presentaties met alle sappige plaatjes. Van infecties en bacteriën moet jij niet veel hebben, zoals jij het omschrijft: borring! Maar van zo'n kinky coccy kan je nog best veel last hebben. Groetjes aan Albert!

Ard Struijs, Struijs, bedankt voor je luisteren oor en adviezen. Jouw deur stond altijd open, daar heb ik dan ook regelmatig gebruik van gemaakt. Ik vond het super tof om met jou samen te werken in de kliniek. Beiden eigenwijs, resulterend in goede discussies. Ik mis de zeven-tweetje momenten. Bedankt Ard.

Ik wil alle stafleden van de Intensive Care Volwassenen (Johan van den Akker, Jasper van Bommel, Jelle Epker, Hilde de Geus, Christine Groeninx, Jubi de Haan, Ben van den Hoven, Can Ince, Mathieu van der Jagt, Erwin Kompanje, Dinis dos Reis Miranda en Robert van Thiel) bedanken voor de ondersteuning tijdens het uitvoeren van mijn onderzoek op de intensive care. Daarnaast wil i k alle stafleden bedanken voor de klinische ervaring die heb ik mogen opdoen op de intensive care van het Erasmus MC.

Verpleegkundigen

Ik wil alle verpleegkundigen van de 3 zuid, 10 zuid en thorax intensive care bedanken voor alle ondersteuning en inzet die zij hebben geleverd voor mijn onderzoek. Het was een enorme klus, waarbij ongeveer 8000 bloedbuizen zijn gecollecteerd door jullie. Dankzij jullie inzet heb ik deze mooie artikelen kunnen maken.

Els Forman

Dag mevrouw! Els, ik wil je bedanken voor alles wat je voor mij hebt gedaan de afgelopen jaren. Daarnaast kon ik altijd bij je terecht om even heerlijk te

mopperen in de zwarte stoel in de hoek. Gelukkig mopperde je soms ook even lekker terug! Bedankt Els.

Afdeling Klinische Chemie
Robert de Jonge, beste Robert, ik wil je bedanken voor het beschikbaar stellen van de laboratorium machines van de afdeling klinisch chemie. In totaal hebben we 8000 bloedbuizen verwerkt tijdens mijn studie, voor mij een enorm aantal, voor het AKC maar een klein topje van de ijsberg. Daarnaast wil ik je bedanken voor het feit dat je mij in contact hebt gebracht met Sysmex Europe. Dit heeft geresulteerd in een enorme impuls voor de opzet van mijn thesis.

Ik wil alle medewerkers van de afdeling klinische chemie van het Erasmus MC Rotterdam bedanken voor hun inzet tijdens mijn onderzoeksperiode. Speciale dank gaat uit naar Rob Brouwer, Monique de Waart en alle dames van het Trial Lab.

Maasstad Ziekenhuis
Servet Duran, beste Servet, ik wil je bedanken voor alle inzet en steun voor mijn onderzoek de afgelopen jaren. Daarnaast wil ik je bedanken voor de gastvrijheid en de mogelijk tot het verrichten van mijn onderzoek in het Maasstad ziekenhuis.

Ik wil alle verpleegkundigen, arts assistenten en stafleden van de Intensive Care van het Maasstad ziekenhuis bedanken voor hun inzet tijdens de studie. Dankzij jullie inzet hebben we een mooi aantal patiënten kunnen includeren in deze multicenter studie.

Tot slot wil ik de mensen van de afdeling klinische chemie en de afdeling medische microbiologie bedanken voor hun inzet tijdens mijn studie. Speciale dank gaat hierbij uit naar Stan Nijkerken en Oscar Pontesili.

Sysmex Europe
Jo Linssen, beste Jo, ik wil je bedanken voor alle steun en inzet tijdens mijn onderzoek. Ongeveer vier jaar geleden zijn wij met elkaar in contact gekomen en heb ik de kans gekregen om met een zeer interessante en nieuwe biomarker te mogen werken. Onze gesprekken over biomarkers en infectieziekten waren altijd

erg interessant en leerzaam, waarbij we vanuit twee verschillende perspectieven de zaken evalueerden. Ik hoop dat we onze samenwerking zo voort kunnen zetten en nog meer mooie onderzoeken kunnen leveren.

Alexandra Maroz, dear Alex, I would like to thank you for all your support during my research period. I really liked our meetings in Hamburrg were you have taken me to some of the best restaurants in Hamburg. I hope we can continue our cooperation and deliver some more interesting articles.

I would like to thank all other employees of Symex Europe who were involved with my research for their effort and advices.

Leon
Lieve Leon, tegen de tijd dat jij dit leest zijn we bijna twee jaartjes samen. De afgelopen twee jaar heb je mij enorm gesteund. Jij zorgde ervoor dat ik op tijd stopte met werken en vooral ook tijd bestede aan het doen van leuke dingen. Je gaf tegengas en steun op de juiste momenten. De afgelopen twee jaar hebben we vele leuke reisjes gemaakt (Barcelona, Valencia, Altea, Cannes, Monaca, Saint-Tropez, Istanbul en zo kunnen we wel even doorgaan) en vele heerlijke barretjes en restaurantjes ontdekt. Ik hoop dat wij samen nog vele reisjes kunnen maken en nog meer nieuwe restaurantjes kunnen ontdekken, dit alles onder het genot van heerlijke wijntjes. Dikke kus.

Ouders
Lieve Pa en Ma, inmiddels zijn we vijf jaar gemopper en getier verder, maar het boekje is dan eindelijk klaar. Jullie staan altijd voor mij klaar en hebben altijd een luisterend oor. Dit heb ik enorm gewaardeerd, want de afgelopen vijf jaar waren niet altijd makkelijk. Tot diep in de late uurtjes werd doorgewerkt, wat mijn humeur niet altijd ten goeden kwam. Ik ben heel erg blij en dankbaar dat ik altijd bij jullie terecht kon en dat jullie ten alle tijden mij steunden. Jullie inzet is dan ook onbeschrijfbaar. Lieve pa het is dan nu eindelijk tijd voor een borrel, proost. Lieve ma, mijn dagelijks gemopper is nu eindelijk voorbij, voortaan kunnen we leuke dingen bespreken aan de telefoon in plaats van mijn gemopper over dat onderzoek aan te horen.